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TERMIS

6TH WORLD CONGRESS

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THE NETHERLANDS

BOOK OF ABSTRACTS

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PREFACE LOCAL ORGANISING COMMITTEE

Dear participants of the 6th World TERMIS Conference 2021,

How we would have loved to meet you all in Maastricht, in a region where three countries meet, characterized by tradition and innovation. The Romans settled this region and for centuries, it was one of the most important political centers of Europe. Even today, Maastricht had an important role in making it possible through the Maastricht Treaty, which still represents today the cornerstone of collaboration, cooperation, and co-development across Europe.

How we would have loved to show you this special region, at the heart of Europe, where the Dutch University of Maastricht, the Belgian Universities of Liege and Leuven and the German RWTH Aachen University have created an epicenter of Tissue Engineering and Regenerative Medicine like only a few in the world. A fertile international ecosystem of academic, industrial, and governmental stakeholders that could be renamed the “Regenerative Valley” of Europe.

And how we would have loved to discuss, argue, network, laugh and celebrate with you during the breaks, evening events and casual meetings in the cafés of this wonderful old city of Maastricht; just what a lively (scientific) society like TERMIS is all about.

We had prepared all this for you and now this little coronavirus has thrown a spanner in the works. Nevertheless, or even more so now, we want to make the World TERMIS Conference a special event for you. An event that is still being held, but online leading us out of the isolation in our institutes back to scientific communing.

The general theme of the congress is “Biologically inspired technology driven regenerative medicine”, which is fully reflected in the 9 parallel thematic sessions. We want to stimulate a fruitful discussion between scientists and clinicians and industry representatives to foster the translation of ground-breaking ideas into clinical practice.

Enjoy the World TERMIS conference 2021…

…and we hope to see you soon face-to-face again!

Lorenzo Moroni ***Liesbet Geris*** ***Stefan Jockenhövel***

PREFACE TERMIS PRESIDENT AND INCOMING PRESIDENT

Dear Participants of the TERMIS World Congress 2021,

On behalf of Geoff Richards, Tony Weiss and the entire governing board, we would like to thank our three organisers, Lorenzo Moroni, Liesbet Geris and Stefan Jockenhoevel for the TERMIS World Congress. It requires large amounts of work to host a TERMIS World Congress physically, but these three also had the extra work to then prepare for a hybrid meeting and unfortunately finally a fully online meeting. Our personal experiences of hosting meetings remind us how much work is involved but we did not have to prepare for a hybrid and/or online meeting. A big thank you to the three of you!

Thank you all who have prepared and submitted abstracts, symposia and workshops, for all those who have taken time to help review these abstracts and to all of you who have supported this congress by both registering and encouraging others to register.

It has been very difficult to run a Society during the pandemic without our usual three physical Chapter meetings and now with the World Congress going virtual, we have not had a face-to-face meeting since Brisbane in October 2019. Our normal income from these meetings was wiped out from one day to another, and we had to work hard to develop an alternative and more robust way to finance the Society that would work in all situations. The Governing Board decided to move to an annual membership structure for TERMIS, as most international societies have, to help our Society survive during difficult times. We therefore thank all of you who have paid your membership fees in 2020 and 2021 and encourage you to pay early in 2022 – these are essential to run the Society. This will allow you to attend all TERMIS conferences (face-to-face and virtual meetings such as webinars) at a special member rate.

In 2022 we will personally try to attend all three TERMIS Chapter meetings in Poland (TERMIS EU), Toronto (TERMIS AM) and Korea (TERMIS AP) and we hope many of you will be participating to help rebuild our in-person networking to strengthen our Society.

Geoff: As my last TERMIS Congress as the Society President I wish you a great online experience and hope to see you face to face in 2022.

Tony: As the incoming President of TERMIS, I welcome you to our World Congress online and look forward to seeing you in person at future meetings.

R. Geoff Richards, President of TERMIS 2019,2020,2021.

Tony Weiss, incoming President of TERMIS.

ABSTRACTS (BY NUMBER)

These are the abstracts as submitted through the website. Last minute changes, title and presenting changes are not always reflected in this file.

Abstract **#1**

ENABLING TECHNOLOGIES FOR DEVELOPMENT OF BIOARTIFICIAL ORGANS

Corresponding author's email: d.stamatialis@utwente.nl

In the coming years, due to the aging of the population and the low availability of donor organs there will be urgent need for bioengineered organs to assist, mimic or replace failing patient organs.
These organs could be artificial, based on novel biomaterials and designs, to assist or mimic a patient organ or bioartificial: combining biomaterials and biologics (cells and tissues) to fully replace failing patient organs. In this talk, we will discuss enabling technologies for development of bioartificial organs with specific attention to bioartificial kidney, bioartificial pancreas.

References

(1) "Innovations in dialysis: Innovations in dialysis membranes for improved kidney replacement therapy", Nature Reviews Nephrology, I. Geremia, D. Stamatialis (2020), 16(10) p550. 2."Frontiers in hemodialysis: Innovations and technological advances", C Basile, A. Davenport, S. Mitra, A. Pal, D. Stamatialis, C. Chrysochou, D. Kirmizis, Artificial organs, 45 (2021) p175.

Abstract **#2**

TRANSLATIONAL SYSTEMS BIOLOGY OF INFLAMMATION AND WOUND HEALING: FROM DATA TO MODELS TO DIGITAL TWINS

Corresponding author's email: vodovotzy@upmc.edu

Properly regulated inflammation is central to homeostasis, but in adequate or overly robust inflammation can lead to disease. Like many biological processes, inflammation and its various manifestations in disease are both multi-dimensional and multi-compartmental. Our group has carried out studies aimed at obtaining high-dimensional, dynamic data on the etiology and progression of various inflammatory processes and diseases in samples derived from cells, animals, and people; creating computational models based on these data as well as on key principles of immunology, as well as developing novel software tools for data aggregation and analysis; and modulating the inflammatory response in an optimal spatial, temporal, and individual- / disease-specific manner. These studies center on various disease states, especially trauma/hemorrhage, infection, wound healing, and liver failure, as well as the application of computational approaches to regenerative medicine, biomarker discovery, and rational drug/device design. Our ultimate goal is to gain a translational systems perspective on the inflammatory response (1,2).

Keywords

Inflammation; Mathematical modeling ; Systems biology

References

1. An GV, Y. Translational Systems Biology: Concepts and Practice for the Future of Biomedical Research. New York, NY: Elsevier; 2014. 2. Complex Systems and Computational Biology Approaches to Acute Inflammation: A Framework for Model-based Precision Medicine. New York, NY: Springer; 2020

Abstract **#3**

RECENT ADVANCE OF TISSUE ENGINEERING TO ENHANCE NATURAL-HEALING POTENTIALS FOR REGENERATIVE MEDICINE

Corresponding author's email: yasuhiko@infront.kyoto-u.ac.jp

A new therapeutic trial based on the natural-healing potential of body itself to induce tissues regeneration and repairing, has been recently expected. To realize this regenerative therapy, there are two approaches of cell transplantation and tissue engineering. Tissue engineering is a biomaterial technology or methodology to artificially create a local environment which enables cells to enhance their proliferation and differentiation for tissue regeneration. If a cell scaffold or a key bio-signaling molecule is supplied to the right place at the right time period or concentration, the body system initiates to physiologically function, resulting in the natural induction of cell-based tissue regeneration.
The biological functions of bio-signaling molecules can be promoted with drug delivery system (DDS) technology. Biodegradable hydrogels enabled the controlled release of various growth factors and chemokines to succeed in the healing potential -based regeneration and repairing of various tissues through the recruitment and activation of cells. This release and/or cell scaffold technologies can be combined with cell transplantation to significantly enhance the therapeutic efficacy in tissue regeneration. The biomaterials technology of regenerative medicine is also applicable to the basic researches of stem cells biology. The further development of stem cells biology will be effective in enhancing the therapeutic efficacy of cell-based tissue regeneration.
In this paper, several applications of DDS and cell scaffold technologies to the tissue regeneration therapy as well as the basic research of stem cells are introduced to emphasize clinical significance of biomaterials technologies in tissue regenerative therapy.

Keywords
tissue engineering; drug delivery system; regenerative medicine

Abstract **#4**

AUTOMATED PROCESS DEVELOPMENT AND MANUFACTURING STRATEGIES FOR CELL AND GENE THERAPIES

Corresponding author's email: q.rafiq@ucl.ac.uk

Genetically-modified cellular immunotherapies, including chimeric antigen receptor T-cells (CAR-T) and natural killer cells (CAR-NK), have generated significant clinical and commercial outcomes due to their unparalleled response rates against cancer. However, the development and manufacture of these advanced therapies face a number of translational bottlenecks that must be addressed to ensure long-term commercial viability.

We have undertaken extensive studies to investigate the impact of key culture parameters on the yield and quality of CAR-T cells, including medium screening studies, medium feeding strategies, dissolved oxygen concentrations, pH and culture mode of operation. The outcome of these studies has been the development of a novel process control strategy that has resulted in a process intensification efficiency of >40% whilst maintaining the critical quality attributes of the CAR-T cells. We have also established a criteria for scaling the process in stirred-tank bioreactors, ranging from 15mL to 1L, resulting in equivalent growth kinetic and cell quality profiles.

This talk will introduce the process control and intensification strategy established to improve CAR-T yield, outlining the importance of monitoring and control capability. The role of manufacturing technologies will be discussed with data presented from a range of expansion platforms. Finally, the talk will outline the challenges of scalable CAR-T manufacture, particularly for allogeneic (universal donor) applications, and present data from 1L stirred-tank bioreactor studies.

Keywords
ATMPs; Cell Therapy; Gene Therapy

Abstract **#5**

DESIGN OF PHYSIOLOGICAL BIOREACTORS THOUGH IMAGE ANALYSIS AND COMPUTATIONAL MODELLING

Corresponding author's email: m.allenby@uq.edu.au

Cell and tissue biomanufacturing currently rely on cultures suspensions which are inherently expensive, time-consuming, and variable in comparison with how tissue products are grown naturally. These inefficiencies have become critical bottlenecks for human cell and tissue biomanufacturing, restricting the future industrial and clinical translation of lab-grown organs. Few human cell culture products deliver sufficient benefit, value, and consistency to offset their high manufacturing costs to produce useful clinical or biomedical solutions. Recent advances in biomedical image analysis and computational modelling can enhance the design and operation of high-efficiency tissue biomanufacturing platforms, as well as the high-content characterisation and monitoring of culture performance to enable bioprocess control, optimisation, and automation. These computational technologies aim to maximize culture outcomes while minimizing variability and process development expense. In this talk, we outline several approaches which harness biomedical imaging and image-based computational models to design, fabricate, and demonstrate efficient and robust human cell and tissue biomanufacturing platforms.

Keywords
Bioreactors; Imaging; Biofabrication

Abstract **#6**

CRITICAL QUALITY ATTRIBUTES OF CARDIOVASCULAR CELLS DIFFERENTIATED FROM HUMAN PLURIPOTENT STEM CELLS

Corresponding author's email: sppalecek@wisc.edu

The process for manufacturing specialized cell types from human pluripotent stem cells (hPSCs) typically involves guiding the cells through developmentally-relevant cell states via temporal presentation of microenvironmental cues in the bioreactor. However, even with precise control over the cell environment, differentiation is prone to cell line-to-line and batch-to-batch variability because of heterogeneity in the cell population. Failed and inefficient manufacturing runs contribute to the high cost of cell therapies. A move toward closed loop cell manufacturing processes with real-time integrated analytics that inform on population composition and state would presumably increase differentiation efficiency and reproducibility. Here I will discuss efforts to discover cell quality attributes during hPSC differentiation to cardiomyocytes through a multi-omics analysis of cell populations and extracellular media at different stages of differentiation. First, we identified that successful vs. failed differentiations, defined as the purity of cTnT+ cardiomyocytes at the end of the differentiation, can be predicted early in differentiation by metabolite profiling of the media. This suggests that failed differentiations can be identified early and stopped or perhaps corrected through process interventions. We also identified metabolomic and proteomic signatures that correlate with cardiomyocyte maturation state, achieved through extended culture. These results suggest that metabolic pathways can be used to identify cardiomyocyte differentiation efficiency and potency metrics, and that metabolic pathways may be useful control points to improve cardiomyocyte manufacturing for in vitro and in vivo applications.

Keywords
Human Pluripotent Stem Cells; Cardiomyocytes; Critical Quality Attributes

Abstract **#7**

SUPRAMOLECULAR DESIGN OF HYDROGEL BIOMATERIALS

Corresponding author's email: mwebber@nd.edu

Host–guest supramolecular recognition affords a useful tool in the physical crosslinking of macromeric precursors to create hydrogels with dynamic properties and tunable functions for a range of biomedical applications. The recognition affinity of certain macrocycle hosts in binding to a suite of different guests offers a tool to rationally design materials from the molecular scale so as to empower specific and tunable functionality. In the context of their use as biomaterials and drug delivery devices, the modularity of these interactions facilitates opportunities to combine multiple bioactivities or therapeutic payloads within a single delivery platform, as well as routes to the facile incorporation of targeting motifs for functional delivery even in the complex biological milieu. A subset of macrocyclic host–guest chemistries are able to achieve affinities approaching that for biotin-avidin, offering a non-covalent approach to enable recognition in complex environments. Whereas conventional ligation reactions can be kinetically limiting, the association of host–guest motifs occurs near the diffusion limit. Accordingly, these interactions form quickly, and at high affinities may have considerable lifetime. In this work, we describe our efforts to use supramolecular chemistry to empower new biomaterials and drug delivery devices. This approach is also useful in the context of protein modification as a means of altering the stability and pharmacokinetics of therapeutic drugs. Finally, we demonstrate the use of supramolecular recognition to facilitate homing and retention of systemically administered small molecules at desired sites in the body, and in the process overcome dilution and physiologic competition to achieve site-specific accumulation.

Abstract **#9**

3D PRINTABLE HYBRIDS FOR ARTICULAR CARTILAGE REGENERATION

Corresponding author's email: julian.r.jones@imperial.ac.uk

There is unmet clinical need for materials that can help torn cartilage regenerate. Our new Bouncy Bioglass (silica/polycaprolactone-polytetrahydrofuran) hybrid materials [1] can be made to have a coefficient of friction matching that of articular cartilage (in media) [2]. Hybrids have co-networks of inorganic and organic components. This gives unprecedented combination of mechanical properties and control of biodegradation. Hybrid inks were 3D printed in log-pile-like architectures and responded well to cyclic loads [1]. We found that the 3D printed scaffolds guide bone marrow stem cells differentiated down a chondrogenic route in vitro, producing articular cartilage-like matrix, rich in Collagen II, Aggrecan and GAG, but only when the pore channel size was 250 microns [3]. When the pore size was larger, Collagen I was prevalent [3]. However, when polycaprolactone control scaffolds where used, Collagen I was also prevalent. Pore architecture, surface chemistry and stiffness therefore affected cellular response. In vivo sheep studies showed similar results, wherein scaffolds with 250 micron pores showed excellent cartilage regeneration after 3 months, while defects remained when scaffolds with 500 micron pores were used.

Keywords

hybrid; 3D printing; Stem cell differentiation

References

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Abstract **#12**

A NOVEL CONCEPT OF CELL MANUFACTURABILITY AND ITS IMPACT FOR AUTOMATED CELL PRODUCTION

Corresponding author's email: KINO-OKA@BIO.ENG.OSAKA-U.AC.JP

Regenerative medicine and cell therapy use the cell transplants which are manufactured, requiring interdisciplinary activities not only from medicinal and biological fields but also from manufacturing field. The 'cell manufacturability', which is defined as capability of cell manufacturing through the processes by bridging between biological and process aspects, is proposed to be the new concept for discipline of cell production [1]. And "Design for cell manufacturability" is to be manufacturing design of cell-based products in such a way that they are easy to manufacture through simple, safe and efficient (cost-saving) process with stable product quality and secure to customer by considering transportation and preparation outside factory, acting practically the system optimisation for the efficiency and stability of the process by understanding the requirement gap between biological and engineering aspects and reducing output fluctuation, This action will lead to cost saving of the products through the process simplification and governing the kinetics of cell behaviour.

Based on the concepts and our back ground for the development of flexible modular plat form [2], we develop the novel automation (machinery) culture system for retina epithelial pigment cells (RPE cells) derived from iPS cells to realise robust processing by measuring the motion. The system performed the successful production of RPE cells, compared with operation by experts in the laboratory. This result concludes the importance of machinery system for the cell manufacturing.

Keywords

Cell manufacturability; Scale-up and Scale-down; Automation system

References

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Abstract **#14**

SUPRAMOLECULAR BIOFABRICATION: FROM MOLECULAR CONTROL TO MACROSCOPIC FUNCTIONALITY

Alvaro Mata[P][1]

[1] *University of Nottingham*

Corresponding author's email: a.mata@nottingham.ac.uk

Nature has evolved to grow and regenerate tissues and organs using self-assembling processes capable of organizing a wide variety of molecular building-blocks at multiple size scales. As the field of biofabrication progresses, it is essential to develop innovative ways that can enhance our capacity to build more complex macroscopic structures using molecular and nanoscale components organized across size scales and in a rational manner. The talk will present our efforts to develop biofabrication processes integrating supramolecular chemistry, structural biology, and engineering principles to create structures with innovative properties such as hierarchical organization, the capacity to grow or heal, tuneable mechanical properties, and selective bioactivity.

Abstract **#15**

MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES: EFFECTIVE MODULATORS OF THE IMMUNE SYSTEM

Bernd Giebel[P][1]

[1] *Instute for Transfusion Medicine, University Hospiatal Essen*

Corresponding author's email: bernd.giebel@uk-essen.de

Extracellular vesicles (EVs), especially harvested from supernatant of human mesenchymal stem/stromal cells (MSCs) provide a novel tool in regenerative medicine and immune therapy. Compared to cellular therapeutics, EV-based therapeutics provide several advantages. Amongst others due to their small size (70-150 nm) they can be sterilized by filtration, lack any endogenous self-replication potential and are much easier to handle than cells. Data will be presented demonstrating the therapeutic potential of MSC-EVs including their immunomodulatory properties. Furthermore, challenges in the field will be discussed

Abstract **#16**

SKIN SUBSTITUTE WITH ARTIFICIAL DERMIS AND GRAFTED KERATINOCYTE SHEETS FOR FULL-THICKNESS BURN WOUNDS

Yosuke Niimi$_P$[1,2], Suzan Alharbi[2], Vsevolod L. Popov[2], Hiroyuki Sakurai[2], Dominique J. Wiener[3], Donald S. Prough[2], Perenlei Enkhbaatar[2]

[1] *Tokyo women's medical university*

[2] *University of Texas Medical Branch*

[3] *College of Veterinary Medicine & Biomedical Science*

Corresponding author's email: niimiyousuke2000@gmail.com

Introduction: The goal of the present study was to compare the efficacy of CEA overlaid on excised full-thickness burn wounds grafted with either AD or cadaver skin in sheep.

Methods: Six full thickness burn wounds (5×5cm) were induced at the dorsum of sheep. After 24 hours, the eschar was excised down to the fascia, and covered with ovine frozen cadaver skin (CS); or AD. The skin were harvested for isolation of autologous keratinocytes to be cultured on temperature-responsive dishes until forming the multilayer sheets. Three weeks after grafting, the keratinocyte sheets were overlaid onto the wounds covered with either CS or AD. The wound epithelialization were assessed for 14 days using intermittent planimetric assay and histological analysis.

Results: Epidermis of grafted CS started rejecting after 10 days, and its complete rejection was observed within 21 days. AD was gradually changed to dermis-like tissue. The dermis thickness was comparable between CS and AD groups at 7, 14, or 20 days. The percentage of wound epithelialized area after keratinocyte sheet grafting was also comparable between CS and AD groups at 7 days (50.1±7.8% vs 54.2±10.5%, p=0.76) or 14 days (91.2±4.1 vs 87.4±5.8%, p=0.60). The transmission electron microscopy analysis revealed no significant difference in the percentage of lamina densa and number of hemidesmosomes between CS and AD groups.

Conclusions: Our results also demonstrate that AD and autologous keratinocyte sheets can substitute autologous skin grafts.

Keywords

Keratinocyte sheet; temperature responsive dish; ovine model

Abstract **#17**

TENDON STRUCTURE FUNCTION RELATIONSHIPS: INJURY AND THE INTERFASCICULAR MATRIX

Hazel Screen$_P$[1]

[1] *Queen Mary, University of London*

Corresponding author's email: h.r.c.screen@qmul.ac.uk

Tendon injury is debilitating and recalcitrant. It is primarily associated with energy storing tendons, likely related to the greater mechanical demands placed on these tendons. However, the mechanobiology of tendon injury remains unclear. The presentation will focus on the structural specialisms associated with tendon energy storage, and how poor specialisation or ageing changes may in turn be associated with increased injury risk.

Our data demonstrate that energy storing tendon specialism is focused in the non-collagenous matrix between the fascicles, termed the interfascicular matrix (IFM). In energy storing tendons, the IFM is elastin- and lubricin-rich, facilitating sliding and recoil between fascicles with increased fatigue resistance [1-3]. The IFM is highly cellular, and is repaired and maintained at a faster rate than the collagen fascicles. It is also the first region of tendon to respond to overload damage, suggesting injury may initiate in the IFM.

Tendons from older, more injury prone individuals show loss of IFM structural specialization and reduced tendon fatigue resistance [4,5]. We also see reduced turnover rate of the IFM suggesting less capacity for IFM renewal [6].

Taken together, our data indicate an active and mechanoresponsive IFM cell population maintaining the heavily loaded IFM region of energy storing tendons in health, and a mechanical and cellular response to overload focused in the IFM and leading to tendon injury.

We now focus our attention towards developing in vivo and in vitro models to explore IFM mechanobiology and potential treatments for tendon injury, in further detail.

References

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Abstract **#18**

ENGINEERING THE CELL-MATRIX INTERFACE - UNDERSTANDING AND GUIDING CELL FUNCTION

Claudia Loebel$_P$[1]

[1] *University of Pennsylvania*

Corresponding author's email: loebelcl@seas.upenn.edu

The native extracellular microenvironment is dynamic, as cells synthesize, assemble, and remodel their surroundings during tissue development, injury, and repair. Biomaterials, such as hydrogels, have evolved as valuable tools to both investigate mechanisms of cell-extracellular matrix (ECM) interactions (e.g., mechanobiology) and to guide desired cell behavior towards the development of new therapies (e.g., tissue repair/regeneration); however, the dynamic nature of the cell-ECM interface has been underappreciated. To address this, we are utilizing metabolic labeling techniques to visualize secreted matrix components to better understand how this nascent matrix influences cellular behavior and we are designing proteolytically degradable and viscoelastic hydrogels that harness dynamic cell-hydrogel interactions. We have used these techniques to explore questions related to cellular mechanosensing in 3D(1), to better understand the evolution of matrix in modifying the cell-hydrogel interface in the engineering of tissues (e.g., cartilage(2)), and to develop microfabricated hydrogel platforms for the culture of organoids towards cellular therapies and as in vitro models of tissue repair. Our evolving understanding of this cell-material interface will not only open up new avenues for engineering functional biomaterials, but will allow us to design better systems for medical therapies.

References

1. Loebel, C., Mauck, R. L. & Burdick, J. A. Local nascent protein deposition and remodelling guide mesenchymal stromal cell mechanosensing and fate in three-dimensional hydrogels. Nature Materials 18, 883-891, 2019; 2. Loebel, C. et al. Metabolic Labeling to Probe the Spatiotemporal Accumulation of Matrix at the Chondrocyte–Hydrogel Interface. Advanced Functional Materials, in press

Abstract **#19**

THE SWEET SIDE OF BIOMATERIALS: CLARIFY THE ROLE OF GLYCANS

Francesco Nicotra[P][1,2]

[1] *University of Milano-Bicocca*

2 IUPAC

Corresponding author's email: francesco.nicotra@unimib.it

The morphology and biomolecular composition of ECM induces cell fate, resulting in tissue regeneration or in pathological events. Glycans are indispensable components of ECMs, to which they provide not only structural and even mechanical properties, but also biological signals. It is interesting to observe how Nature has orchestrated cell homing, proliferation and morphogenesis exploiting the two major classes of biomolecules, protein and glycans, with synergistic but also quite different roles. Structural features, swelling properties, capacity to perform stronger or weaker interactions, with lower or higher specificity, are alternatively governed by glycans versus proteins. Clarify the contribution of glycans is even more challenging due to the heterogeneity and dynamism of their composition in the ECM components and at the cell surfaces. Evidences indicate that aging, diet, environmental factors and pathologies significantly influence the glycosignature, in a cause-effect relationship not yet clarified. In order to spread light in this very intrigued matter, strategies are needed to generate ECMs functionalized with different glycans, and study the specific cell responses. The functionalization approaches to conjugate biomaterials with glycidic cues and to crosslink polysaccharide to proteins or synthetic polymers will be described and their role to induce specific cell fate analyzed.

References

Sampaolesi S., Nicotra F., Russo L., Glycans in Nanomedicine, Impact and Perspectives. Future Medicinal Chemistry, 11(1), 43, 2019

Abstract **#20**

ENGINEERED SKIN GRAFT WITH STROMAL VASCULAR FRACTION CELLS ENCAPSULATED IN FIBRIN–COLLAGEN HYDROGEL: A CLINICAL STUDY FOR DIABETIC WOUND HEALING

Mahsa Mollapour Sisakht[P][1,2]

[1] *Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Department of Biochemistry, Erasmus University Medical Center, Rotterdam, the Netherlands*

Corresponding author's email: m.molapoursisakht@erasmusmc.nl

Despite the abundance of skin substitutes in the worldwide market, major hurdles in developing more complex tissues include the addition of skin appendages and vascular networks as the most important structure. The aim of this research was a clinical feasibility study of a novel prevascularized skin grafts containing the dermal and epidermal layer using the adipose stromal vascular fraction (SVF)-derived endothelial cell population for vascular network regeneration. Herein, we characterized hydrogel with emphasis on biological compatibility and cell proliferation, migration, and vitality. The therapeutic potential of the prevascularized hydrogel transplanted on five human subjects as an intervention group with diabetic wounds was compared with nonvascularized skin grafts as the control on five patients. Wound planimetric and biometric analysis was performed using a Mann–Whitney nonparametric t-test ($p \leq .05$). The fibrin–collagen hydrogel was suitable for skin organotypic cell culture. There was a significant ($p \leq .05$) increased in skin thickness and density in the vascular beds of the hypodermis measured with skin scanner compared with that in the control group. No significant macroscopic differences were observed between the intervention and control groups ($p \leq .05$). In summary, we report for the first time the use of autologous dermal–epidermal skin grafts with intrinsic vascular plexus in a clinical feasibility study. The preliminary data showed that SVF-based full-thickness skin grafts are safe and accelerate the wound healing process. The next stage of the study is a full-scale randomized clinical trial for the treatment of patients with chronic wounds.

Keywords

skin tissue engineering; stromal vascular fraction; wound healing

References

Nilforoushzadeh MA, Sisakht MM, Amirkhani MA, et al. Engineered skin graft with stromal vascular fraction cells encapsulated in fibrin-collagen hydrogel: A clinical study for diabetic wound healing. J Tissue Eng Regen Med. 2020; 14(3):424-440. doi:10.1002/term.3003.

Abstract **#21**

STEM CELLS AND BIOMATERIALS FOR THE REGENERATIVE MEDICINE OF INTERVERTEBRAL DISC: A PARADIGM SHIFT IN SPINE SURGERY

Jerome Guicheux[P][1]

[1] *INSERM U1229-RMeS, Regenerative Medicine and Skeleton research Centre, Nantes University & Hospital, ONIRIS, Nantes, France.*

Corresponding author's email: jerome.guicheux@inserm.fr

Degenerative disc disease (DDD), shares a lot of its pathogenic mechanisms (tissue degeneration, inflammation..) with osteoarthritis (OA) and is one of the major causes of low back pain (LBP). Currently, LBP is primarily managed by pharmacological treatments and if unsuccessful by surgical procedures (spine fusion or arthroplasty). To clinically address LBP earlier in the degenerative cascade of IVD, biology-inspired regenerative strategies could offer less invasive and etiological alternatives to spinal reconstructive surgery (1). Considering their tissue regenerative abilities, anti-inflammatory and immunomodulatory properties and their promising clinical outcomes in knee OA, the intradiscal injection of mesenchymal stem/stromal cells (MSC) have been contemplated with a growing interest.

In this context, we will first share our view of the mesenchymal stromal cells (MSC)-based therapeutic approaches that have been preclinically developed and, for some of them, clinically transferred in patients with DDD-associated LBP (2). Then, we will comment on the recent biomaterial-assisted MSC therapies that recently enter the preclinical and clinical scene and discuss whether injectable biomaterials that could be used as cell carriers and percutaneously transplanted into degenerated IVDs may be a relevant therapeutic strategy. We will also discuss whether induced pluripotent stem cells (iPS) could be a relevant cell source for the production of regenerative IVD cells (3) and whether iPS-derived disc cells may warrant clinical use.

Finally, we will present some recent perspectives (3D Bioprinting, extracellular vesicles, endogenous regeneration (4), annulus repair (5)..) and whether these concepts may pave the way of future therapeutic developments for DDD-associated LBP.

References

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Abstract **#22**

BIOMATERIAL AND TISSUE ENGINEERING STRATEGIES FOR DISC REGENERATION AND REPLACEMENT

Conor T Buckley$_P$[1,2,3]

[1] *Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, The University of Dublin, Dublin, Ireland*

[2] *Advanced Materials and Bioengineering Research (AMBER) Centre, Royal College of Surgeons in Ireland & Trinity College Dublin*

[3] *Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, 121/122 St. Stephen's Green Dublin 2, Ireland*

Corresponding author's email: conor.buckley@tcd.ie

Low back pain resulting from intervertebral disc (IVD) degeneration is a global problem. In the field of disc degeneration, several cell-based technologies have reached the clinical trial stage [1,2]. While these are exciting times in the field, it is unclear whether cell-based approaches will lead to improved patient outcomes within reasonable timeframes. An important factor that may limit the potential of all cell-based regeneration strategies is the intrinsic harsh microenvironment that exists within the degenerated intervertebral disc, which is characterised by reduced oxygen, reduced glucose, matrix acidity and inflammation [3]. Biomaterials, and in particular injectable hydrogel-based systems, have played an important role over the past decade overcoming some of the issues associated with cell leakage, by providing a protective niche during intradiscal delivery as well providing important physicochemical cues to transplanted or resident cells. Advances have also been made in designing and developing composite biomaterials to direct specific biological responses or by enhancing biomechanical properties using fibre-like materials. More recently, advances have been made in tissue engineering the entire disc including the annulus fibrosus and cartilage endplates. While this ambitious goal is still in its infancy, it is a significant advancement and refinements are likely to emerge in the coming years.

This talk will highlight some of the important advances in biomaterials and existing challenges in the field of intervertebral disc regeneration and discuss emerging technologies and strategies that include advanced biomaterials, cell priming, predictive in silico modelling and biofabrication technologies.

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Abstract **#23**

THE POTENTIAL OF THE MESENCHYMAL STROMAL CELL SECRETOME IN EQUINE REGENERATIVE MEDICINE

Gerlinde Van de Walle[P][1]

[1] *Cornell University*

Corresponding author's email: grv23@cornell.edu

The secretome is defined as the global group of bioactive factors secreted by cells which regulates a variety of biological and physiological process, thus making it a clinically relevant source to identify potential therapeutics. To explore the potential of the equine MSC secretome as a safe and effective therapy in equine medicine, our lab studies its functional effects in the context of wound healing. The prevalence of impaired cutaneous wound healing in horses is high, and treatment is difficult and often ineffective. In addition to bioactive molecules with regenerative properties, we also identified secreted factors with antimicrobial properties against bacteria that commonly infect wounds, including the methicillin-resistant Staphylococcus aureus (MRSA), both in planktonic form and when organized in biofilms. For the latter, we found that equine MSC secrete cysteine proteases that destabilize MRSA biofilms, thereby increasing the efficacy of antibiotics that were prior tolerated by the biofilms. Collectively, our results demonstrate the potential of the equine MSC secretome as a promising new therapy in equine wound management.

This work has been funded by an Agriculture & Food Research Initiative Competitive Grant no. 2017-05722 from the USDA National Institute of Food and Agriculture and by the Harry M. Zweig Memorial Fund for Equine Research.

Abstract **#24**

REGENERATIVE CELL THERAPY FOR OSTEOARTHRITIS

Gunil Im[P][1]

[1] *Dongguk University*

Corresponding author's email: imgunil@hanmail.net

When cell therapy was first reported to treat cartilage defects and osteoarthritis, an optimistic view prevailed which held that implanted cells could be incorporated into defects and regenerate AC. The main focus was on how to ensure that the implanted stem cells possess the full properties of articular chondrocytes. As it became evident that almost all IA-administered stem cells rapidly undergo apoptosis and that their principal mode of action is paracrine, two strategies rose up to help enhance the effects of cell therapeutics: 1) enhance the survival of stem cells by pretreatment with factors known to promote cell survival or by administration of stem cells in physical states that favor longer-term survival, such as the spheroid form or encapsulated in a hydrogel, and 2) apply stem cells in a high concentration and prevent dispersal into the joint using a bioadhesive .Given that the mechanisms underlying necrosis versus survival of implanted stem cells are not well-established, future studies should focus on how the fate of IA-administered stem cells is affected by factors such as the physical status of the cells, mode of implantation, and adjuvant biomaterials.

Abstract **#25**

PROGENITOR CELLS OF THE INTERVERTEBRAL DISC FOR TISSUE ENGINEERING – FAKE OR SOON A REALITY FOR THE CLINICS?

Benjamin Gantenbein$_P$[1,2]

[1] *Department for BioMedical Research (DBMR) University of Bern*

[2] *Department for Orthopedics & Traumatology, Insel Hospital, University of Bern*

Corresponding author's email: benjamin.gantenbein@dbmr.unibe.ch

Stem cell therapy for the intervertebral disc (IVD) is highly debated but holds great promises. From previous studies, it is known that notochordal cells are highly regenerative and may stimulate other differentiated cells to produce more matrix [1,2]. Lately, a particular tissue-specific progenitor cell population has been identified in the centre of the intervertebral disc (IVD [3]. The current hope is that these nucleus pulposus progenitor cells (NPPC) could play a particular role in IVD regeneration.

Current evidence confirms the presence of these cells in murine, canine, bovine and in the human fetal/surgical samples [4]. Noteworthy, one of the main markers to identify these cells, i.e., Tie2, is a typical marker for endothelial cells. Thus, it is not very clear what their origin and their role might be in the context of developmental biology. In human surgical specimens, their presence is, even more, obscured depending on the donor's age and the particular condition of the IVD [3, 4] and other yet unknown factors

Here, I revisit the recent literature on regenerative cells identified for the IVD in the past decades. Current evidence how these NPPC can be isolated and detected in various species and tissues will be recapitulated. Future directions will be provided on how these progenitor cells could be used for regenerative medicine and tissue engineering.

Acknowledgements

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Abstract **#26**

ENGINEERING BIOMIMETIC FIBER SCAFFOLDS: FROM MIMICKING THE EXTRACELLULAR MATRIX TO NEURONAL AXONS

Sing Yian Chew$_P$[1]

[1] *Nanyang Technological University*

Corresponding author's email: sychew@ntu.edu.sg

Fiber constructs closely mimic the size-scale and architecture of the natural extracellular matrix. When presented to appropriate cell types, engineered fibers may also pose as artificial axons. These platforms represent a unique class of materials in regenerative medicine and non-viral drug/gene delivery. Fibrous topography can direct cell fate. Combined with the incorporation of gene silencing molecules, such as small non-coding RNAs, these scaffolds provide synergistic topographical and biochemical cues to cells. Here, we will present our recent findings on the roles of fiber-mediated mechanotransduction on oligodendrocyte differentiation and myelination, as well as gene silencing on nerve regeneration and remyelination, using spinal cord injury as a proof-of-principle.

Abstract **#27**

BIOMATERIALS THAT ENABLE AND DRIVE SCAFFOLD-FREE TISSUE FORMATION

Eben Alsbeerg$_P$[1]

[1] *University of Illinois at Chicago*

Corresponding author's email: ealsberg@uic.edu

Scaffold-free cultures of cells can mimic immature condensates that initiate many developmental and healing processes. Providing specific soluble signals, such as growth factors, exogenously in tissue culture media to regulate cell behavior and promote new tissue formation may be limited by transport issues, lack of spatial control over signal presentation, and required repeated dosing. The first part of this talk will be a report on systems that incorporate biomaterial microparticles containing bioactive signals within scaffold-free cell cultures, permitting localized spatiotemporal control over the presentation of these regulatory cues to the cells to engineer a variety of tissues. Three-dimensional bioprinting has been pursued as a method of building functional scaffold-free tissue constructs with sophisticated geometries without an intervening scaffold that can interfere with critical cell-cell interactions. However, to date it has not been possible to print individual cells directly, as preculturing was required to first form cell aggregates or strands prior to printing. The latter part of this talk will cover the first bioprinting platform using single cells alone, without a macromer solution, as bioink that can maintain printed geometries. A shear-thinning and self-healing biomaterial support bath was engineered to permit high-resolution printing into precise geometries, maintenance of cell viability, cellular condensation formation and long-term culture of constructs for development of engineered tissues. Collectively, these platforms may advance cellular condensation-based regenerative medicine strategies, enhance drug-screening capabilities and provide new tools for addressing questions in developmental biology.

Abstract **#28**

CLINICAL TRANSLATION OF ANGIOGENIC GENE THERAPY

Ilia Bozo$_P$[1,2], Igor Plaksa[3], Alexey Drobyshev[4], Vladimir Komlev[5], Artur Isaev[3], Roman Deev[3,6]

[1] *Histograft, LLC*

[2] *A.I. Burnazyana Federal Medical Biophysical Center, FMBA of Russia*

[3] *PJSC "Human Stem Cells Institute"*

[4] *A.I. Evdokimov Moscow State University of Medicine and Dentistry*

[5] *A.A. Baykov Institute of Metallurgy and Materials Science of the RAS*

[6] *I.I. Mechnikov North-Western State Medical University*

Corresponding author's email: bozo.ilya@gmail.com

Gene therapy provides a wide range of opportunities to improve treatment of patients with different diseases and even change the face of medicine. In 2011, a gene-therapeutic drug based on plasmid DNA encoding VEGFA gene was approved for treatment of patients with chronic lower limb ischemia ("Neovasculgen", HSCI, Russia). To date, thousands of people who suffered from this disease have been treated in Russia, with significant clinical improvement. In a long-term clinical study, the therapeutic effect persisted 5 years post the injection course (NCT03068585). A second clinical trial showed efficacy of Neovasculgen for diabetic foot syndrome treatment (NCT02538705) where wound healing was observed in 65.7% of patients with chronic ulcerative defects and the target limb salvage rate was 84%.Angiogenic effect induced by plasmid DNA carrying VEGFA has been utilised in development of gene-activated bone substitute based on octacalcium phosphate scaffold ("Histograft", Histograft, Russia). We conducted an open-label non-randomised clinical trial. 20 patients with alveolar ridge atrophy and jawbone defects were enrolled and underwent bone grafting. There were no adverse events or serious adverse events during the clinical trial or the follow-up period (more than 4 years). Newly formed tissues had a density similar to native bone (908.13±114.40 HU) that ensured the patients to receive dental implants. The histological analysis showed that the bone grafting zone consisted of newly formed bone tissue with some fragments of the gene-activated bone substitute partially-resorbed and integrated with native bone without fibrous tissue in between. To date, numerous patients underwent bone grafting with Histograft.

Abstract **#29**

APPLICATION OF MSCS-BASED THERAPY IN THE TREATMENT OF OSTEOARTHRITIS

Jianhao Lin[P][1,2]

[1] *Peking University People's Hospital*

[2] *Peking University*

Corresponding author's email: linjianhao@pkuph.edu.cn

Osteoarthritis (OA) is a slowly progressive disease which ultimately leads to degeneration of articular cartilage. Mesenchymal stem cells (MSCs) have been tested over the last decade as a potential regenerative therapy for treat- ing OA. Preclinical and clinical studies have recently demonstrated some favorable effects on cartilage repair when MSCs were injected into OA joints in animal models and in humans. In our studies, we firstly confirmed the early effects of MSCs on the progress of OA. We, then, investigated an improved strategy for cell delivery to OA joint through the use of three-dimensional (3D) microcryogels. Recently, we found that the 3D microcryogels with MSCs assembled into a tissue construct, which has been used in cartilage regeneration.

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Abstract **#30**

EX SITU AND IN SITU IMAGING OF MG IMPLANTS TO MODEL DEGRADATION AND TISSUE REMODELLING

Regine Willumeit-Römer[P1], Berit Zeller-Plumhoff[1], Jalil Nourisa[1]

[1] *Helmholtz Zentrum Geesthacht, Metallische Biomaterialien*

Corresponding author's email: regine.willumeit@hzg.de

Magnesium based implants are a new class of degradable metallic medical devices. Their performance and degradation properties depend on a plethora of factors. To understand the complex interaction of the material with the environment, ex situ and in situ techniques like synchrotron radiation based µCT can be applied to study the degradation of the material over time. This data can then be used to model the degradation process which in turn can be coupled to a model which describes the biochemical effects of Mg and alloying elements on tissue regeneration. Based on this approach a clearer view on the dynamic interaction between metal and tissue will be presented.

Abstract **#31**

MODULATING IMMUNITY WITH MATERIALS

David Mooney[P][1]

[1] *Harvard University*

Corresponding author's email: mooneyd@seas.harvard.edu

Immune cells play multiple roles in tissue regeneration, but strategies to effectively manipulate these cells and their functions in the body are limited. We are creating biomaterials capable of concentrating, interrogating, and manipulating immune and stem cells to promote regeneration.

Abstract **#32**

PCP/VANGL2 SIGNALLING REGULATES WNT CYTONEMES

Steffen Scholpp[P][1], Lucy Brunt[1]

[1] *Living Systems Institute, University of Exeter*

Corresponding author's email: s.scholpp@exeter.ac.uk

After secretion, developmental signals known as morphogens must travel distances to form a concentration gradient that the responding tissue uses to acquire positional information. The role of morphogen transport in this process is the subject of intense debate. Wnt proteins regulate developmental processes, tissue regeneration and stem cell maintenance. It has been postulated that Wnt/beta-catenin signalling form concentration gradients across responsive tissues and act as morphogens. However, little is known about the transport mechanism for these lipid-modified signalling proteins in vertebrates.

Here we show that Wnt proteins are transported on short, actin-based cytonemes to contact responding cells and activate signalling. We further demonstrate that the transmembrane protein Vangl2 in concert with the receptor Ror2 activates the beta-catenin independent PCP pathway to regulate the formation of these signalling cytonemes. We show that PCP/Vangl2 signalling in the Wnt producing cells controls the effective signalling range of Wnt in zebrafish embryos, in the mouse intestinal crypt and in human gastric cancer cells.

We conclude that cytoneme-based trafficking is fundamental for Wnt signalling in vertebrates.

Abstract **#33**

IMAGING MASS SPECTROMETRY OF THREE DIMENSIONAL CELL CULTURES AND ORGANOIDS

Amanda Hummon[P][1]

[1] *The Ohio State University*

Corresponding author's email: hummon.1@osu.edu

Three dimensional cell cultures are attractive models for biological research. They combine the flexibility of cell culture with some of the spatial and molecular complexity of tissue. For example, colon cancer cell lines form spheroids, in vitro mimics of poorly vascularized tumors. The spheroids are composed of a central necrotic core, a middle quiescent layer and an outer proliferative layer of cells, similar to a rapidly growing colon tumor. Our laboratory has characterized the distribution of endogenous proteins via MALDI imaging mass spectrometry in colon spheroids and determined that the molecular gradients correlate with the pathophysiological changes in the structure. We have also developed an approach to employ 3D cell cultures to evaluate the penetration of compounds into cellular masses. Most novel drugs are initially evaluated with 2D cultures before moving directly to costly animal studies. 3D cultures provide an ideal testbed to minimize these studies. Working with the chemotherapeutics oxaliplatin and irinotecan, our data supports differential penetration of these clinically relevant drugs. We are employing microfluidic devices to enable dynamic dosing, thus investigating the pharmacokinetics and pharmacodynamics of chemotherapy regimes in these attractive model systems. We have also extended our imaging mass spectrometry platforms to the study of patient and mouse-derived organoids, examining the distribution and metabolism of drugs compounds in these clinically relevant specimens.

Abstract **#34**

A BIOMIMETIC MACROPOROUS HYBRID SCAFFOLD WITH SUSTAINED DRUG DELIVERY FOR ENHANCED BONE REGENERATION

Seunghun S Lee[P][1], Matthias Santschi[1], Stephen J Ferguson[1]
[1] *Institute for Biomechanics, ETH Zurich, Zurich, Switzerland*
Corresponding author's email: seunglee@ethz.ch

Bone regeneration is a highly complex physiological process regulated by several factors. In particular, bone-mimicking extracellular matrix and available osteogenic growth factors such as bone morphogenetic protein (BMP) have been regarded as key factors to induce bone regeneration. In this study, we developed a biomimetic hybrid scaffold (CEGH) with sustained release of BMP-2 that would result in enhanced bone formation. This hybrid scaffold, composed of BMP-2 loaded cryoelectrospun poly ε-caprolactone (PCL) (CE) surrounded by a macroporous gelatin/heparin cryogel (GH), is designed to overcome the drawbacks of the relatively weak mechanical properties of cryogels and poor biocompatibility and hydrophobicity of electrospun PCL. The GH component of the hybrid scaffold provides a hydrophilic surface to improve the biological response of the cells while the CE component increases the mechanical strength of the scaffold, to provide enhanced mechanical support for the defect area and a stable environment for osteogenic differentiation. After analyzing characteristics of the hybrid scaffold such as hydrophilicity, pore difference, mechanical properties and surface charge, we confirmed that the hybrid scaffold group shows enhanced cell proliferation rate and apatite formation in simulated body fluid. Then, we evaluated drug release kinetics of CEGH and confirmed the sustained release of BMP-2. Finally, the enhanced osteogenic differentiation of CEGH with sustained release of BMP-2 was confirmed by Alizarin Red S staining, and real-time PCR analysis.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 812765.

Keywords
Hybrid Scaffold; Bone regeneration; Controlled drug release

Abstract **#35**

RESETTING THE EPIGENETIC CLOCK: DNA METHYLATION CHANGES IN REPROGRAMMING AND DIFFERENTIATION OF IPSCS

Wolfgang Wagner[P][1], Julia Franzen[1], Yang Han[1]

[1] *RWTH Aachen Medical School*

Corresponding author's email: wwagner@ukaachen.de

Aging of the organism as well as culture expansion of primary cells is associated with highly reproducible DNA methylation (DNAm) changes at specific sites in the genome – therefore these processes can be tracked by different epigenetic predictors. The so called “epigenetic clocks” provide biomarkers to either gain insight into aging or for quality control of cells in culture. In this presentation, I will show ongoing work to further optimize epigenetic clocks with barcoded bisulfite amplicon sequencing (1, 2). Upon aging/passaging the DNAm patterns of neighboring CpGs become more complex, while they do not appear to be coherently regulated. During reprogramming into induced pluripotent stem cells (iPSCs) the age-associated as well as long-term culture-associated DNAm changes are reversed simultaneously with pluripotency-associated DNAm changes. Interestingly, iPSC-derived cells remain epigenetically rejuvenated for age-associated changes, whereas upon differentiation particularly the culture-associated DNAm changes are again gradually acquired. Chromatin immunoprecipitation demonstrated that CTCF binds particularly in the vicinity of genomic regions that become hypomethylated upon aging, whereas it is rather enriched at regions that become hypermethylated during long-term culture. Thus chromatin conformation seems to have a different impact on epigenetic clocks for aging and in vitro expansion.

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Abstract **#36**

IMPLANTABLE THERAPEUTIC RESERVOIR SYSTEMS FOR DIVERSE CLINICAL APPLICATIONS IN LARGE ANIMAL MODELS

Garry P. Duffy[P][1,2]
[1] *National University of Ireland Galway*
[2] *CURAM SFI Centre for Medical Devices*
Corresponding author's email: garry.duffy@nuigalway.ie

Significant potential has been attributed to regenerative medicine approaches, specifically stem cell technologies, to treat a diverse array of pathologies, however the functional outcomes of such approaches have been limited as studies have unanimously shown poor retention/survival of delivered cells. A delivery system that facilitates regional and repeated delivery to target tissues can provide enhanced clinical efficacy of cell therapies when localized delivery of high doses of cells are required.
We have developed a regenerative reservoir platform (Regenervoir) for use in small and large animal models, with relevance to cardiac, abdominal, and soft tissue pathologies. Regenervoir incorporates multiple novel design features essential for clinical translation, with a focus on scalability, mechanism of delivery, fixation to target tissue, and filling/refilling with a therapeutic cargo, and is demonstrated in an array of clinical applications that are easily translated to human studies.

Abstract **#37**

THE DEVELOPEMENT AND TRANSLATION OF TISSUE ENGINEERED CONSTRUCTS FOR USE IN CONGENITAL HEART SURGERY

Christopher Breuer[P][1]

[1] *Nationwide Children's Hospital*

Corresponding author's email: christopher.breuer@nationwidechildrens.org

Congenital cardiac anomalies represent the most common birth defect, affecting approximately 1% of live births. Despite advances in surgical and medical management, these conditions remain a leading cause of death in newborns. A significant source of postoperative morbidity and mortality in this patient population arises from the use of prosthetic materials, in the form of vascular grafts, cardiovascular patches, and replacement heart valves, which are associated with an increased risk of thromboembolic events, poor durability, susceptibility to infection, and lack of growth capacity. In contrast, tissue engineering offers a promising strategy for creating improved biomaterials to repair or replace tissues in children undergoing congenital heart surgery. Our research team pioneered the development and clinical translation of tissue engineering technologies to create improved vascular grafts, cardiovascular patches, and replacement heart valves for the surgical repair of congenital cardiac anomalies. Using the tissue engineered vascular graft (TEVG) as the archetype for tissue engineered cardiovascular constructs, we demonstrated the feasibility of using this technology. Our tissue engineering approach uses a biodegradable scaffold upon which an individual's cells can be seeded. Neotissue forms as the scaffold degrades, ultimately creating a living autologous structure called a neovessel, which is devoid of synthetic components and possesses an ability to repair, remodel, and grow. In this presentation, we will describe the development, translation, and refinement of the TEVG. Having viable cardiovascular constructs with growth potential enables reconstructive procedures at an early age, mitigates the potential for additional surgeries due to overgrowth, and could improve patient outcomes.

Abstract **#38**

CURRENT STRATEGIES FOR STRUCTURAL AND PHYSIOLOGICAL MATURATION OF IN-VITRO ENGINEERED HUMAN MUSCLE TISSUES

Hironobu Takahashi[P][1], Fumiko Oikawa[2], Naoya Takeda[2], Tatsuya Shimizu[1]
1 Tokyo Women's Medical University
2 Waseda University
Corresponding author's email: takahashi.hironobu@twmu.ac.jp

Since skeletal muscles contribute to metabolic, neuromuscular, and dystrophic disorders, tissue-engineered muscle tissue models will become a powerful tool to elucidate the mechanisms of muscle diseases and facilitate the development of new drugs for their treatments. Several research groups including us have reported innovative strategies to artificially regenerate the native-like aligned structure of muscle tissues. On the other hand, engineered human muscle tissues remain difficult to be matured significantly in vitro, compared with rodent muscle tissues. In order to hurdle this issue, some defined culture methods have been recently developed. Our group has also reported a new culture method for the production of human muscle tissue construct with biomimetic features.1 Whereas these techniques would be applied for the studies of muscle physiology and pathology in human body, they are ill-equipped to pursue new findings in the study of neuromuscular diseases including a process requiring the development of neuromuscular junction (NMJ). Since muscle wasting and weakness in motor neuron diseases (MNDs) arise predominantly from the loss of normally function motor neurons, some research groups are currently focusing on the development of NMJs in tissue constructs. Particularly, when coupled with the ability to obtain iPS cells from patients with MNDs such as amyotrophic lateral sclerosis (ALS) and spinal muscle atrophy (SMA), the tissue engineering technology will make it possible to produce diseased NMJ systems in vitro. In conclusion, these newly developed methods exhibit potential to produce muscle tissue models which will become a new platform for the biological studies of neuromuscular pathophysiology.

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Abstract **#39**

PERSONALIZED TUMOR ORGANOIDS FOR ADVANCEMENT OF CANCER TREATMENTS

Shay Soker[P][1,2]
[1] *Wake Forest School of Medicine*
[2] *Wake Forest Institute for Regenerative Medicine*
Corresponding author's email: shaysoker@yahoo.com

Major challenges in cancer therapy are to determine if a local disease will progress to a malignant phenotype, and how to best 'match' treatments to an individual patient. For many years, basic cancer research and anti-cancer drug development utilized primary cancer cells and cell lines cultured in vitro on tissue culture plastic dishes. Cell culture is a poor analogue of tumor growth in vivo, in part because it doesn't replicate the microenvironment of a tumor, a complex space typified by stromal cells, ECM components, and a cocktail of signaling factors. Newly developed bioengineered tissue platforms, such as 3D orgaonids, open new opportunities for tumor modeling, especially incorporating the complex cellular and physical tumor microenvironment that are known to drive cancer cells in a specific manner. For example, the tumor stroma can activate or inactivate cancer-related pathways, alter ECM components, thereby making migration more or less difficult, as well as secrete signaling factors that guide cancer cells in a multitude of ways. A significant advantage of the tumor organoids is that they can be derived from individual patients and enable personalized testing of drug response, including immunotherapies. Furthermore, personalized tumor organoids have the potential to serve as a drug discovery platform and identification of new therapeutic targets, based on individual pathology, mutational profiles and tumor cell clonality.

Abstract **#40**

FETAL SECRETOME – A NOVEL THERAPEUTIC STRATEGY FOR CARTILAGE REGENERATION

Iris Ribitsch$_P$[1], Lukas Janker[2], Rupert Mayer[2], Monika Egerbacher[1], Simone Gabner[1], Maciej Kandula[3], Sinan Gültekin[1], Andrea Bileck[2], David Kreil[3], Christopher Gerner[2], Florien Jenner[1], Gerjo van Osch[4]

[1] *Vetmeduni Vienna*

[2] *University of Vienna*

[3] *BOKU Vienna*

[4] *EMC Rotterdam*

Corresponding author's email: iris.ribitsch@vetmeduni.ac.at

Recapitulation of developmental processes is discussed as a potential key to cartilage regeneration.
The aim of the presented study was therefore to test the effect of fetal secretomes on inflamed chondrocytes in vitro.
A comprehensive in vitro experiment was carried out employing gene expression analysis (sheep specific whole genome microarrays), protein secretion analysis (label free bottom-up shot gun proteomics by high resolution orbitrap mass spectrometry), proliferation assays, wound healing assays and beta-Galactosidase assays for cell senescence to characterize the effect of fetal secretomes (obtained from fetal chondrocytes and fetal MSCs) on adult injured chondrocytes (inflamed with 10ng IL1beta and TNFalpha per ml of medium). Secretomes of three fetal donors (for chondrocytes and MSCs) where pooled and tested on inflamed adult chondrocytes obtained from three different individuals.
We further compared the secretome composition and the response of the treated chondrocytes to key factors of fetal regeneration identified in previous studies. In these previous studies standardized cartilage lesions were surgically induced in adult (aged 2-4 years) and fetal sheep (80 days gestation). Time points for sampling were chosen according to the stages of healing (inflammatory phase, reparative phase, remodelling phase). Samples were compared for protein secretion and on the histologic level with special emphasis on extracellular matrix composition, collagen organization and collagen types, qualitative and quantitative composition of growth factors and cytokines, inflammatory response, chemotaxis, cell proliferation, MMPs and migration as well as senescence.
Tremendous differences between adult and fetal regeneration were observed with regard to inflammation-related processes and extracellular matrix remodelling.

Abstract **#41**

HUMAN PLACENTA MATERIAL-BASED BIOMATERIALS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Johannes Hackethal[P][1,2], Anna Maria Weihs[3,4], Alexandra Hofer[5], Lisa Karner[1], Magdalena Metzger[1], Peter Dungel[1], Simone Hennerbichler[4,6], Heinz Redl[1], Andreas Herbert Teuschl[4,3]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in AUVA Trauma Research Center, Vienna, Austria*

[2] *THT Biomaterials GmbH, Vienna, Austria*

[3] *Department of Life Science Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria*

[4] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[5] *Research Area Biochemical Engineering, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria*

[6] *Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria*

Corresponding author's email: office@THT-Biomaterials.com

INTRODUCTION: Human extracellular matrix (ECM) proteins are regarded as the perfect scaffold material for the manufacturing of medicinal products.[1] Numerous publications have shown that human ECM goes along with a better performance in cell culture and clinical applications when compared to animal ECM.[2] Human placenta is globally available and a constant source for industrial processing. The aim of this project was to establish a human ECM protein-based platform technology from placenta for cell culture applications.

MATERIAL/METHODS: Human ECM proteins were extracted using enzymatic or non-enzymatic strategies. Collagen-1 (COL1/3) was extracted using pepsin.[3] Laminin-111 (pLm111) and a basement-protein rich human placenta substrate (hpS) were extracted using Tris-NaCl buffers.[4,5] All extracted proteins were extensively characterized and used in different 2D or 3D in vitro experiments using HUVEC, hepatozytes, fibroblasts, or other cells.

RESULTS: Significant differences in cell viability, growth rate, or phenotype were observed between animal-based proteins and human-based proteins (COL1/3, pLm111, hpS), favoring the use of human-materials based for cell culture applications.

DISCUSSION/CONCLUSION: The source of biomaterials significantly influences the results of cell culture experiments. Human ECM proteins support the “3R” criteria in science and enable a better quality when compared to animal materials. Human ECM proteins could bring more precision into TERM, refine in vitro diagnostics or drug screening applications and by that reduce the drug development time in medicine. However, more experiments have to be performed, to assess the full potential of human ECM proteins for TERM.

Acknowledgements

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Abstract **#42**

TACKLING IMPLANT-ASSOCIATED INFECTIONS: TO PREVENT OR TO TREAT?

Rikke Meyer[P][1]

[1] *Interdisciplinary Nanoscience Center, Aarhus University*

Corresponding author's email: rikke.meyer@inano.au.dk

Implant-associated infections become chronic when bacteria form biofilms in which the bacteria exist are encased in a self-produced extracellular matrix that protects them from phagocytosis. Biofilms harbor persister-cells, which are dormant or slow-growing bacteria that tolerate extreme levels of antibiotics. These cells revive and cause the infection to relapse when antibiotic treatment is terminated. If the implant cannot be surgically replaced, the prognosis for some implant-associated infections is as poor as some of the worst cancers.

Faced with the failure of antibiotic treatment, decades of research has been devoted to understanding how bacteria attach to implants and form biofilms, and whether biofilm infections can be prevented by intercepting bacterial attachment. However, there is little evidence to suggest that the impressive results from anti-adhesive materials studied in the lab, will translate into lower infection rates in the clinic. Development of more effective treatments may therefore be more likely to succeed in tackling implant-associated infections. Such treatments rely on either high local doses of antibiotics, or development of entirely new drugs that effectively kill bacteria in a dormant state.

This keynote lecture gives an overview and discuss developments in anti-adhesive materials to prevent implant-associated infections, and the strategies for novel drugs or drug-delivery methods that eradicate the infection when prevention fails.

Abstract **#43**

OSTEOCONDUCTION AND BONE AUGMENTATION: WHEN 3D-PRINTED DESIGNS MEET BONE BIOLOGY

Franz E Weber[P][1]

[1] *University of Zurich/Oral Biotechnology & Bioengineering*

Corresponding author's email: franz.weber@zzm.uzh.ch

In the last decades, advances in bone tissue engineering mainly based on osteoinduction and on stem cell research. Only recently, new efforts by others and us focused on the micro- and nanoarchitecture needed to improve and accelerate bone regeneration. By the use of additive manufacturing, libraries of diverse microarchitectures were produced and tested to identify the ideal pore size or rod distance for osteoconduction to occur. Presently, we try to elucidate the dependency of osteoconduction on microporosity and expand our view on micro- and nanoarchitecture of bone substitutes for optimal bone augmentation.

For the production of scaffolds, we applied for titanium-based scaffolds selective laser melting and for ceramics the CeraFab 7500 from Lithoz, a lithography-based additive manufacturing machine. As in vivo test model, we used a calvarial defect and a bone augmentation model in rabbits. The histomorphometric analysis showed that bone formation was significantly increased with pores between 0.7-1.2 mm in diameter. Moreover, microporosity appeared to be a strong driver of osteoconduction and influenced osteoclastic degradation. Best microarchitecture for osteoconduction and bone augmentation are not identical.

In essence, additive manufacturing enabled us to generate libraries of microarchitectures to search for the most osteoconductive microarchitecture and the ideal microarchitecture for bone augmentation purposes. Moreover, additive manufacturing appears as a promising tool for the production of personalized bone tissue engineering scaffolds to be used in cranio-maxillofacial surgery, dentistry, and orthopedics.

Keywords

osteoconduction; vertical bone augmentation; microarchitecture

Abstract **#45**

IN VITRO TISSUE RECONSTRUCTION USING DECELLULARIZED PERICARDIUM CULTURED WITH CELLS FOR LIGAMENT REGENERATION

Tsuyoshi Kimura[1], Mika Suzuki[1], Yoshihide Hashimoto[1], Hironobu Takahashi[2], Tatsuya Shimizu[2], Naoko Nakamura[3], Akio Kishida[1]

[1] *Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University*

[2] *Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University*

[3] *Department of Bioscience and Engineering, Shibaura Institute of Technology*

Corresponding author's email: kimurat.mbme@tmd.ac.jp

Decellularized tissue is an extracellular matrix obtained by removing cells from living tissue and is expected to be one of candidate biomaterials because of its high biocompatibility and high functionality. One of the problems is that the recellularization of a decellularized tissue is slow when used as a substitute tissue because infiltration of cells into the interior is extremely slow and difficult. Therefore, in this study, as one solution, a 3D tissue with cells was developed by re-cellularization and fabrication of the decellularized pericardium (d-pericardium). Porcine pericardium was treated with high hydrostatic pressure and then washed to remove cellular debris. The decellularization was confirmed by DNA quantification and HE staining. For SEM observation of the surface of d-pericardium, the wavy fibers with the diameter of about 0.5µm were observed and the fibers were aligned with same direction. Cells (C2C12 or hMSC) were seeded on the d-pericardium. Cells were adhered along with the direction of fibers on the d-pericardium and proliferated on the d-pericardium keeping the direction. The recellularized pericardium was cylindrically rolled so that the cell extending direction was parallel to the long axis direction of the cylinder and cultured for 3 days under static and flow conditions. The cells were still observed between the decellularized pericardium. The tensile strength of the 3D reconstructed tissue was similar to that of porcine anterior cruciate ligament. These results suggest that the ligament-like tissue was developed by the cylindrical formation of the recellularized d-pericardium.

Abstract **#46**

DYNAMIC RECIPROCITY IN SUPRAMOLECULAR BIOMATERIALS: TOWARDS RESPONSIVE BIOMATERIALS

Joyce Brouns[P][1,2], Patricia Dankers[1,2]

[1] *Eindhoven University of Technology, Department of Biomedical Engineering*

[2] *Institute of Complex Molecular Systems (ICMS), Eindhoven University of Technology*

Corresponding author's email: j.e.p.brouns@tue.nl

Dynamic reciprocity (DR) refers to the bidirectional interaction between cells and their extracellular matrix. One example of DR is the activity between matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). In pathophysiological conditions, the balance between MMP and TIMP activity can be shifted, and excessive MMP activity can cause maladaptive changes to the tissues [1]. The aim of this project is the exploration of dynamic reciprocity in synthetic solid materials via feed-back controlled mechanisms.

We make use of supramolecular materials that are based on supramolecular motifs that allows polymers to self-assemble into supramolecular structures using hydrogen bonding [2]. These supramolecular motifs were coupled to MMP cleavable peptides and MMP inhibitor peptides and processed into a supramolecular scaffold. We showed that the MMP cleavable peptide can be cleaved both in solution and from surface. Moreover, the MMP inhibitor peptide could inhibit the MMP activity even when incorporated into a supramolecular surface. Next steps involve the incorporation of bioactive molecules in the system that can be cleaved from the surface upon different cellular cues. In this way, a biomaterial can be developed that respond to cellular cues (enzymes) by releasing molecules. This process can be inhibited by the release of inhibitor peptides. In this way, a biomaterial can be developed that shows a true interaction with the host tissue, being able de adapt and respond to cellular cues.

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Abstract **#48**

MODULAR HYDROGELS REVEAL THAT ILC1 DRIVE EPITHELIAL AND MATRIX REMODELLING IN HUMAN IPSC-DERIVED INTESTINAL ORGANOIDS

Eileen Gentleman[1], Geraldine Jowett[1], Tracy Yu[1], Emily Read[1], Michael Norman[1], Joana Neves[1]

[1] *King's College London*

Corresponding author's email: eileen.gentleman@kcl.ac.uk

Type-1 innate lymphoid cells (ILC1) are enriched in patient mucosa with active inflammatory bowel disease (IBD), but the impact of this accumulation remains elusive. We established co-cultures of murine small intestinal organoids (SIO) with ILC1, and human iPSC-derived intestinal organoids (HIO) with patient ILC1. Transcriptomic analysis of SIO co-cultures revealed that IFNγ sensitises epithelial cells to Fas-mediated apoptosis. However, ILC1 also drive expansion of the epithelial stem cell crypt through p38γ phosphorylation and aberrant Cd44v6 expression, which is unexpectedly regulated by ILC1-derived TGFβ1, not IFNγ. We next established that human ILC1 also secrete TGFβ1, and drive CD44v6 expression in both HIO epithelium and the surrounding mesenchyme, though this phenotype is only recapitulated by ILC1 from patients with active gut inflammation. As TGFβ1 is a master regulator of fibrosis, we next characterised the ability of ILC1 to regulate matrix remodelling. We created modifiable PEG hydrogels that cross-link quickly but at low stiffnesses and harnessed this platform to perform microrheology and atomic force microscopy on encapsulated HIO. We show that ILC1 drive matrix stiffening and degradation, which we posit occurs through a balance of MMP9 degradation and TGFβ1-induced fibronectin deposition. Our synthetic organoid co-culture system enabled us to tease apart an important role for intestinal ILC1 in epithelial and matrix remodelling, which may drive either wound healing or fibrotic pathologies in IBD. Moreover, this controlled 3D microenvironment provides a broader platform for dissecting interactions between complex tissues and rare cell subtypes in development and disease.

Keywords

Organoid; Hydrogel; Disease modelling

Abstract **#49**

GRADIENT POROUS DESIGN OF ORTHOPAEDIC IMPLANT BASED ON ADDITIVE MANUFACTURING

Changning Sun[1], Ling Wang[1], Dichen Li[1]

[1] *State Key Laboratory for Manufacturing System Engineering, School of Mechanical Engineering, Xi'an Jiaotong University, P.R. China*

Corresponding author's email: sun.cn@xjtu.edu.cn

Porous gradient structure is able to provide both mechanical strength and bone ingrowth of implant by adapting the porosity gradient. The aim of this study was to develop a general methodology for the design of customized gradient porous implant and to validate the osteointegration of a new type of porous structure. In macro level, the optimal distribution of mechanical properties of the implant was obtained in line with the design criterions of safety and stability, under the control of self-developed algorithms in FE model. In micro level, the relationship between geometric parameters and the equivalent mechanical properties of micro representative volume element (RVE) was investigated by finite element analysis and validated via mechanical tests. The osteointegration of a new kind of unit cell, body-centered cubic (BCC), was validated using animal experiments of rabbit model. The gradient porous implant was established by assembling all the RVEs automatically according to the functional relationship between elastic modulus and geometrical parameters. A hip implant was taken as an example. The gradient porous structure of a hip stem was automatic modelled with BCC unit based on optimized elastic modulus distribution. There were two aspects of contribution of this study: In micro level, the mechanical properties and osteointegration of micro porous unit cell were investigated. In macro level, a general methodology for the design and modelling of gradient porous implant was provided.

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Abstract **#50**

HIGH-DEFINITION BIOPRINTING ENABLED BY LIGHT

Aleksandr Ovsianikov[P][1,2]
[1] *3D Printing and Biofabrication Group, TU Wien (Vienna, Austria)*
[2] *UpNano GmbH (Vienna, Austria)*
Corresponding author's email: aleksandr.ovsianikov@tuwien.ac.at

Light is a powerful tool for pattering biomaterials and engineering complex 3D cell culture matrices. It is therefore not surprising, that lithography-based 3D printing methods and according materials are enjoying rapid adoption in the field of biofabrication and tissue engineering.
Multiphoton lithography (MPL) is relying on the nonlinear absorption of femtosecond laser pulses [1]. MPL is outstanding among light-based approaches since it can produce features much smaller than a single mammalian cell. Usually MPL is perceived as a method capable of high-resolution volumetric structuring via photopolymerization of the material. However, MPL can also be used to induce photodegradation or spatially resolved covalent binding of specific molecules in the volume of the sample [2]. Herewith, MPL is a versatile tool when it comes to precise engineering of 3D biomimetic cell culture matrices. Furthermore, we have recently demonstrated direct embedding of cells via MPL using specialized gelatin based bioink formulations, paving the way for High-Definition Bioprinting [3]. Our results demonstrate a wide range of exciting applications, from controlling morphology of stems cells in 3D to modelling barrier tissues in vitro and patterning of hierarchical vascular networks using co-culture of endothelial and stem cells.
In this talk, the recent progress on MPL development will be presented. Current state of the art, challenges and future perspectives will be discussed.

Invited talk at the "Smart biomaterials and dynamic biofabrication processes powered by light" Symposium, chaired by Riccardo Levato & Michiya Matsusaki.

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Abstract **#51**

SEGMENTAL BONE RECONSTRUCTION BY OCTACALCIUM PHOSPHATE COLLAGEN COMPOSITES WITH TERIPARATIDE

Shinji Kamakura$_P$[1], Keiko Matsui[1], Tadashi Kawai[2], Yushi Ezoe[1], Toshiki Yanagisawa[1], Tetsu Takahashi[1]

[1] *Tohoku University*

[2] *Iwate Medical University*

Corresponding author's email: kamakura@tohoku.ac.jp

Octacalcium phosphate and collagen composite (OCPcol) demonstrated superior bone regeneration with angiogenesis (1, 2), and have been commercialized recently in Japan (3). Teriparatide (TPTD) is a bioactive recombinant form of parathyroid hormone that is approved for osteoporosis treatment. And OCPcol with a local single administration of TPTD enhanced bone repair of a rodent critical-sized bone defect (4, 5). Because mandibular bone reconstruction after segmental resection is a key clinical problem, it was examined whether single-dose local administration of OCPcol with TPTD can affect recovery after this procedure. OCPcol was prepared, and a commercially available hydroxyapatite and collagen composite (HAPcol) was used as a control. A 15 mm length segmental bone defect was made in the mandibular region of male beagle dogs. The experimental animals were divided in four groups. OCPcol treated with TPTD (OCPcol+TPTD), OCPcol, HAPcol treated with TPTD (HAPcol+TPTD), or HAPcol was implanted into the defect. The radiopaque areas of the implanted site were measured and statistically analyzed, and histological examination was performed after 6 months. The value of radiopaque area in total region of OCPcol+TPTD was highest, followed in order by OCPcol, HAPcol+TPTD, and HAPcol, and that of OCPcol+TPTD was significantly higher than that of HAPcol+TPTD or HAPcol. All segmented mandibles of OCPcol+TPTD and a part of those of OCPcol were bridged with newly formed bone, whereas no bone bridges were observed in HAPcol+TPTD or HAPcol. These results suggested that OCPcol treated with TPTD enabled bone reconstruction after segmental mandibular resection more than other three groups.

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Abstract **#52**

REGENERATIVE MEDICINE APPROACHES TO INTERVERTEBRAL DISC, IMPORTANCE OF PATHOLOGY

Christine Le Maitre[P][1]
[1] *Sheffield Hallam University*
Corresponding author's email: c.lemaitre@shu.ac.uk

Low back pain is the leading cause of morbidity worldwide and yet most therapies fail to target the cause and are purely symptomatic or end stage surgical options. Intervertebral disc degeneration is associated with approximately 40% of low back pain cases and thus a target for potential regeneration. Intervertebral disc degeneration is a catabolic process caused by altered cell behaviour and tissue biomechanics, leading to a harsh environment for potential cell therapies. A viscous cycle of degeneration, whereby cellular changes impact on matrix composition and biomechanical environment interact, leading to acceleration of degeneration. Degeneration leads to ingrowth of nerves into the normally aneural disc and increased synthesis of pain sensitizers leads to increased pain formation. To generate a successful regeneration strategy for the intervertebral disc this harsh environment must be considered. Here, the pathogenesis of disc degeneration will be discussed to highlight the importance of understanding the disease processes prior to developing regenerative approaches. The potential influence of the pathogenic processes which lead to disc degeneration will be discussed in light of regenerative approaches under development. Cellular, gene and biomaterial approaches for regeneration will be highlighted and their potential to halt the viscous cycle of degeneration enabling regeneration.

Abstract **#53**

INJECTABLE HYDROGEL FOR DISC REGENERATION STUDY OF INJECTABLITIY AND MECHANICAL PROPERTIES IN WHOLE HUMAN INTERVERTEBRAL DISCS

Christine Le Maitre[P][1], Joseph Snuggs[1], L Beckman[2], Hosni Cherif[2], Simon Partridge[1], Lee Breakwell[3], Ashley Cole[3], Neil Chiverton[3], Chris Sammon[1], Lisbet Haglund[2]

[1] *Sheffield Hallam University*

[2] *Montreal General Hospital*

[3] *Sheffield Hospitals NHS Trust*

Corresponding author's email: c.lemaitre@shu.ac.uk

INTRODUCTION: We have previously reported the development of an injectable hydrogel (NPgel), which has the potential to deliver patients own stem cells, via small bore needles, decreasing damage to the annulus fibrosus. NPgel drives stem cell differentiation to NP cells, and can inhibit the degenerate niche. However, clinical success of NPgel is dependent on the capacity to inject NPgel into naturally degenerate human discs, restore mechanical function to the IVD, and prevent extrusion during loading. Here, we investigated the injection of NPgel containing IOHEXOL to enable visualization during injection into human cadaveric discs and performed extrusion testing to determine whether NPgel was retained in the disc.
METHODS: Cadaveric discs were prepared with intact vertebral bone, X-ray images were captured from transverse and sagittal planes together to determine disc height. Discs were pre-warmed to 37ºC prior to mechanical analysis, discs were loaded under simulated walking conditions to calculate Moduli. Following initial testing discs were injected with NPgel containing IOHEXOL using fluoroscopy to visualize injection and disc height measured. Moduli measurements were determined post injection prior to ultimate strength testing to determine whether any NPgel extruded.
RESULTS: NPgel (600-1000µl) was easily injected into cadaveric discs where it filled cracks and fissures. NPgel injection resulted in significant increase in disc height and youngs moduli, furthermore NPgel was not extruded during failure testing.
CONCLUSIONS: These results support the use of NPgel as an injectable therapy for regeneration of disc degeneration.

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Abstract **#54**

A FIBER-ORIENTED MUSCLE MODEL FOR PREDICTING THE SOFT TISSUE DEFORMATION DURING MUSCLE CONTRACTION

Lei Tang[P][1], Cunqing Shan[1], Ling Wang[1], Dichen Li[1], Senay Mihcin[2]

[1] *State Key Laboratory for Manufacturing System Engineering, School of Mechanical Engineering, Xi'an Jiaotong University, 710054, Xi'an, ShaanXi, China*

[2] *Mechanical Engineering Department, Izmir Institute of High Technology, Urla, Izmir, Turkey*

Corresponding author's email: tanglei@stu.xjtu.edu.cn

Effective designing of rehabilitation apparatus with high comfort and functionality depends upon the accurate characterization of the shape of the residual limb as well as its volume and shape fluctuations. The active behavior of skeletal muscles, which plays an important role in the interfacial biomechanics of human-machine interaction, is not considered in the current design processes of the rehabilitation apparatus. In this study, a three-dimensional finite element (FE) model of the human thigh was proposed to simulate the soft tissue deformation caused by muscle contraction. In this model, the muscle model was composed of personalized muscle geometries and parametric muscle fibers. For obtaining the nearest computational deformation response to the real muscles, the model parameters of the muscle fibers were adjusted iteratively according to the differences between the deformation distributions obtained from the numerical calculation and the experimental measurements. The FE model with optimized parameters proved to be effective in accurately predicting the dynamic deformation responses of the soft tissue. The results demonstrated that the average errors for soft tissue deformation in the expanding and shrinking regions of the thigh were 1.34mm and 2.27mm, respectively. For different gaits, the average difference in the equivalent volume and cross-sectional area changes were less than 0.83% and 1.86%, respectively. The soft tissue deformation data can be used in data-driven computational algorithms for the design of prosthetic sockets or orthoses as well as other wearable technologies mechanically interfacing with the skin.

Abstract **#55**

EFFECTS OF EXTRACORPOREAL SHOCKWAVE THERAPY ON PERIPHERAL NERVE REGENERATION AFTER AUTOGRAFT REPAIR OF THE RAT MEDIAN NERVE

Johannes Heinzelp[1,2,3], Claudia Keibl[2,3], Viola Oberhauser[2,3], Nicole Swiadek[2,3], Barbara Schädl[2,3], Michaela Sima[2,3], Helen Frick[2,3], Gregor Längle[2,3], Cosima Prahm[1], Jonas Kolbenschlag[1], David Hercher[2,3]

[1] BG Clinic Tübingen, Department for Hand-, Plastic-, Reconstructive and Burn Surgery, Schnarrenbergstraße 95, 72076 Tübingen, Germany

[2] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Donaueschingenstraße 13, 1200, Vienna, Austria

[3] Austrian Cluster for Tissue Regeneration

Corresponding author's email: hannes.heinzel@gmx.de

Introduction: Recent studies investigating the effects of extracorporeal shockwave therapy (ESWT) on peripheral nerve regeneration in the rat have shown proregenerative effects of this non-invasive treatment approach. However, results of ESWT treatment after conduit repair of peripheral nerve defects have remained unstudied yet. It was the aim of this study to investigate the effects of ESWT treatment on nerve regeneration following conduit repair of the rat median nerve.
Methods: In 123 male Lewis-rats a 7-mm segment of the right median nerve was removed and immediately reconstructed using either 1) an autologous nerve graft 2) a muscle-in-vein-conduit 3) a chitosan-conduit or 4) a silk fibroin-conduit. Half of the animals of each group (n= 10 - 16) were treated once with ESWT postoperatively. Functional recovery was evaluated until 12 weeks postoperative by means of the grasping test, computerized gait analysis and electrophysiological evaluations.
Results: Despite some positive trends, there were no significant effects of ESWT on grasping strength. Electrophysiological investigations revealed superior results of the autologous graft + ESWT group in comparison to both muscle-in-vein groups ($p<0.05$) and both silk fibroin groups ($p<0.05$). There were no significant effects of ESWT detectable via computerized gait analysis.
Conclusion: Despite partial positive effects of ESWT on electrophysiological properties we were unable to detect significant effects on functional recovery. This study's ambivalent results could on the one hand be related to the animal model used, on the other hand the mechanisms, optimal application form as well as application frequency of ESWT remain to be elucidated.

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Abstract **#56**

3D HYDROGEL MICROENVIROMENTS OF GELATIN AND HYALURONIC ACID FOR LIVER TISSUE ENGINEERING

Gloria Gallego-Ferrer[P1,2], Julio Rodríguez-Fernández[1], Emma García-Legler[1], Sandra Clara-Trujillo[1,2], M. Teresa Donato[3,4], Manuel Salmeron-Sanchez[5,1,2], Laia Tolosa[3,2]

[1] *Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Valencia, Spain*

[2] *Biomedical Research Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Valencia, Spain*

[3] *Experimental Hepatology Unit, Health Research Institute La Fe (IIS La Fe), Valencia, Spain*

[4] *Department of Biochemistry and Molecular Biology, School of Medicine, University of Valencia, Valencia, Spain*

[5] *Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, United Kingdom*

Corresponding author's email: ggallego@ter.upv.es

Regenerative therapies aim to enhance liver tissue repair and regeneration in different liver diseases. Hydrogels have been proposed to improve cell engraftment and survival. This study aims to evaluate the ability of biomimetic injectable hydrogels to promote liver functionality of encapsulated human hepatocytes and enhance in vivo outcomes in an animal model of acute liver failure.

Hydrogels of gelatin (GEL), hyaluronic acid (HA) and a GEL/HA mixture 20/80 were prepared by enzymatic crosslinking. GEL gelation is faster (6 min) than in HA (23 min) or in the mixture (13 min). The stiffer hydrogel was HA with a shear storage modulus of 509 ± 136 Pa, whereas GEL was very soft, 35 ± 7 Pa. GEL/HA had similar mechanical properties as HA, 459 ± 103 Pa. Ureogenesis, cytocrome P450 activity and albumin secretion of human hepatocytes encapsulated within GEL/HA hydrogel were enhanced, compared to monolayer cultures. Lyophilized sponges of GEL/HA seeded with human hepatocytes were implanted in mice with acute liver failure. The survival of animals implanted with sponges containing human hepatocytes was 100%, while in the non-transplanted animals it was 65%. Transaminases levels decreased significantly in transplanted animals compared to non-transplanted animals, indicating a positive effect of transplanted cells on recovery from injury.

GEL/HA hydrogel is a good candidate for liver regeneration.

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Keywords

gelatin; hyaluronic acid; human hepatocytes

Abstract **#57**

HUMAN 3D ORGANOTYPIC CULTURE SYSTEMS TO MODEL TISSUE DEVELOPMENT, PHYSIOLOGICAL FUNCTION, PATHOLOGICAL CHANGES AND RESPONSE TO DRUGS

Ivan Martin$_P$[1]

[1] *Dept. of Biomedicine, University Hospital Basel*

Corresponding author's email: ivan.martin@usb.ch

Culture models based on human cells and capturing features of tissue physiology and pathology are key to advance fundamental understanding of development, to learn how to regulate homeostatic processes, and to test new therapies in degenerative or malignancy settings. This lecture will provide an overview of past and ongoing work of the group on the generation and use of 3D human organotypic bioreactor-based models. Following establishment of mesenchymal cell-based stromal tissues and embedding of vascular structures using perfusion flow, the miniaturization into microfluidic models will be introduced to increase throughput, include mechanical loading features and identify compounds regulating lineage commitment and rescuing from catabolic processes.Generated tissues will also be validated as stromal niches to sustain hematopoietic stem cells growth and differentiation, thus recapitulating in vitro the structure and function of bone marrow organs. Introduction in the system of malignant hematopoietic cells (e.g., from patients with myeloproliferative neoplasm or acute myeloid leukemia) will exemplify the possibility to model bone marrow pathologies in personalized settings.Finally, use of cancer cell lines and human primary tumor specimens will illustrate that the culture system allows to engineer tumor models and to generate patterns of response to drugs or immunotherapy strategies, which cannot be mimicked by 2D cultures or by more simple spheroids. Ongoing studies are targeting the connection of bone/bone marrow with tumor models to investigate metastatic processes of prostate and breast tumor cells.

Abstract **#58**

BIOPRINTING OF A RENAL IN VITRO MODEL

Gabriele Addario[P][1], Sonja Djudjaj[2], Silvia Fare'[3], Peter Boor[2,4,5], Lorenzo Moroni[1], Carlos Mota[1]

[1] *Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue regeneration Department, 6229 ER Maastricht, The Netherlands*

[2] *Institute of Pathology, RWTH University of Aachen, Aachen, Germany*

[3] *Politecnico di Milano, Dipartimento di Chimica, Materiali e Ingegneria Chimica, 20131 Milano, Italy*

[4] *Division of Nephrology, RWTH University of Aachen, Aachen, Germany*

[5] *Electron Microscopy Facility, RWTH University of Aachen, Aachen, Germany*

Corresponding author's email: g.addario@maastrichtuniversity.nl

It is estimated that 10% of the worldwide population suffers from chronic kidney disease (CKD) with a rising tendency. [1] Patients with CKD have limited treatment options and novel therapies that could halt or even reverse the progression of CKD are urgently needed. [1] Bioprinting is considered one of the most promising approaches to generate novel 3D in vitro models and organ-like constructs to investigate underlying pathomechanims of kidney diseases. [2-5].

This study aimed at establishing a method to isolate primary renal cells in an easy and reproducible way. These cells were used in a new bioprinting platform laying the foundation for the development of a 3D renal tubulointerstitium model for in vitro studies. Primary murine tubular (pmTECs), endothelial and fibroblast cells were successfully isolated, but further optimization is required for the culture and expansion of primary endothelial cells. Therefore, an endothelial cell line (HUVECs) and pmTECs were combined with polysaccharide biomaterial ink solutions and processed with a microfluidic 3D bioprinter, leading to high cell viability and metabolic activity. Core-shell bioprinted constructs with HUVECs and pmTECs were manufactured mimicking tubules. In conclusion, microfluidic bioprinting strategy could be used to build a novel 3D kidney in vitro model.

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Abstract **#59**

THE MORPHOLOGICAL WONDERLAND OF MELT ELECTROWRITING

Paul D. Dalton[P][1]

[1] *University of Oregon*

Corresponding author's email: pdalton@uoregon.edu

Melt electrowriting (MEW) uses an applied voltage to prevent a fluid jet from undergoing Plateau–Rayleigh instabilities, rather than initiate electrical instabilities (i.e. whipping), required for electrospinning (1). The result is well-positioned fibers in the low-micron scale that, when layered in 3D, result in a high porosity scaffolds or a reinforcing structure for matrices/bioinks. Since biofrabrication is focused on recapitulating hierarchical features of tissue, MEW is a high-resolution 3D printing tool that permits such design at the microscale. There have been several breakthroughs in MEW over the past few years including the use of dynamic electrical fields (2) and microscale-layer shifting (3) that increases the build height and geometric design options respectively. Subtle microscale shifts in layer by layer design can have substantial impacts in macroscopic mechanical properties. For the purpose of translating biomaterials to the clinic, the melt processing of medical-grade materials makes a compelling case (4), and many biomaterial polymers are processable with MEW. Most, if not all of the 3D printed implants used in the clinic to date are solvent-free and based on raw materials made under good manufacturing practice (GMP) - conditions. Furthermore, MEW partners well with digitization through online monitoring allowing validation of manufacture. After a decade of development with multiple biocompatible and biodegradable polymers, including as composite materials, MEW is poised to become a leading technology to produce advanced biomaterials with hierarchical structures.

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Abstract **#60**

THE LONG AND WINDING ROAD TO CARDIAC REGENERATION

Thomas Eschenhagen[P][1]

[1] *University Medical Center Hamburg Eppendorf, Institute of Experimental Pharmacology and Toxicology, German Centre for Cardiovascular Research*

Corresponding author's email: t.eschenhagen@uke.de

Loss of cardiac muscle cells, e.g. by ischemia or overload, represents a key reason of heart failure with reduced ejection fraction, a leading cause of morbidity and mortality worldwide. In contrast to other organs, the postnatal heart possesses only minimal regenerative capacity. It lacks progenitor cells, and cardiomyocytes exit the cell cycle shortly after birth and do not re-enter after injury. Thus, any loss of cardiomyocytes is essentially irreversible, a conclusion already drawn >century ago. Experimental studies from the early 2000´s claiming the opposite and reporting the formation of new heart muscle from bone marrow cells led to the initiation of thousands of experimental and clinical cell therapy studies, but were eventually found to be fraudulent. It is now clear that injected or infused non-myocyte cells do not form new myocytes and that the beneficial effects consistently observed in preclinical animal models are the consequence of paracrine or immunological effects. True cardiac regeneration has thus remained an unfulfilled promise. Yet, through a clearer comprehension of signaling pathways that regulate the cardiomyocyte cell cycle and advances in pluripotent stem cell technology, strategies have evolved that demonstrate the potential to generate new myocytes and thereby fulfill an essential central criterion for heart repair. The three major strategies include in situ reprogramming on fibroblasts into myocytes, the induction of cardiomyocyte proliferation, and the transplantation of cardiomyocytes generated pluripotent stem cells either by direct injection or beating heart muscle patches, a technique the author´s group contributed to that will be discussed at more detail.

Abstract **#61**

BIOMATERIAL-GUIDED GENE THERAPY FOR CARTILAGE REPAIR

Magali Cucchiarini[P][1]

[1] *Center of Experimental Orthopaedics, Saarland University Medical Center Kirrbergerstr. Bldg 37, D-66421, Homburg/Saar, Germany*

Corresponding author's email: mmcucchiarini@hotmail.com

Articular cartilage defects are highly prevalent problems in orthopaedics and may lead to osteoarthritis if left untreated in patients. While various options are available in the clinics, none can defintively and permanently restore an original, hyaline cartilage in its structure and mechanical functions in cartilage lesions. Gene therapy is a powerful tool to enhance the processes of cartilage repair over prolonged periods of time upon the delivery of chondroreparative gene sequences in sites of cartilage injury. Yet, such a strategy remains challenging and may be hindered in vivo by a number of obstacles to effective and durable gene transfer, including physical barriers (synovial fluid), biological barriers (pro-inflammatory mediators), neutralization (pre-existing humoral responses against viral gene vectors), and unwanted gene vector dissemination to non-target sites. The controlled delivery of gene vectors via highly compatible materials (biomaterial-guided gene therapy) is a promising approach to support the sustained and localized release of gene treatments in a spatiotemporally precise fashion, reducing vector dissemination and a loss of the therapeutic gene product, and to mask viral epitopes from possible neutralization (1). This strategy has a strong potential to improve cartilage repair in vivo and may be safely translated into clinical application in a near future.

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Abstract **#62**

TOWARDS A BIOINSPIRED CONTROL OF THE PANCREATIC TUMOUR MICROENVIRONMENT: INCORPORATION OF INTERSTITIAL FLOW ON A MULTICELLULAR SCAFFOLD BASED PANCREATIC CANCER MODEL

Priyanka Gupta[P][1], Pedro Pérez-Mancera[2], Hemant Kocher[3], Andrew Nisbet[4], Giuseppe Schettino[5,1], Eirini Velliou[1]

[1] *University of Surrey, UK*

[2] *University of Liverpool, UK*

[3] *Queen Mary University, UK*

[4] *University College London, UK*

[5] *National Physical Laboratory, UK*

Corresponding author's email: priyanka.g.gupta@ucl.ac.uk

INTRODUCTION: Tissue Engineering has enabled an accurate, reproducible low cost control of various features of the tumour microenvironment (TME), which is not possible with current preclinical models [1-6]. To that end, we have developed a poly urethane (PU) scaffold based model of pancreatic cancer which can be cultured for several weeks and is appropriate for therapeutic assessments [3,4]. Furthermore, we have recently advanced the model via incorporation of biological complexity. More specifically, we developed hybrid scaffolds with tailored extracellular matrix (ECM) for different cells (cancer, stellate, endothelial) of the TME [6]. This static model captures different levels of desmoplasia, i.e., a hallmark of pancreatic cancer, and is functional for several weeks without the need of re-suspension, unlike existing pancreatic cancer models.The aim of this work was to advance our multicellular hybrid static scaffold with interstitial flow. Vascularisation levels differ from patient to patient and can affect metastasis as well as the efficiency of drug delivery [1]. METHODS: Multicellular PU scaffolds [4,5,6] were placed in a bioreactor and perfusion cultures took place for more than 4 weeks. Various in situ assays for monitoring the cell viability, spatial organisation and ECM production were carried out at specific time points throughout the culture period.RESULTS: Addition of interstitial flow in our multicellular model resulted in discernible changes, in comparison to static scaffolds in terms of cellular growth, matrix production and cell migration. Our developed model is a novel, advanced, high throughput tool that can be used for personalized studies of pancreatic cancer.

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Abstract #64

DESIGNING THE MICRO- TO MACRO-ENVIRONMENT OF CELLS FOR TISSUE ENGINEERING APPLICATIONS

João F. Mano$_P$[1]

[1] *University of Aveiro*

Corresponding author's email: jmano@ua.pt

Sophisticated strategies have been employed to encapsulate cells and bioactive molecules in micro-sized matrices using distinct biomaterials, to be explored in cell and tissue engineering. Hydrogels are adequate matrices for 3D culture of cells – examples of natural-based hydrogels with adequate mechanical and biological properties are shown. Using bioinspired strategies basic units of hydrogels can be processed in mild conditions, permitting the encapsulation of living cells and other biological cargo with high efficiency. By using spherical templates containing cells followed by adequate coating procedures it is possible to produce liquefied capsules that may entrap viable cells. The presence of solid microparticles with controlled mechanical properties inside such capsules offers adequate surface area for adherent cell attachment increasing the biological performance of these hierarchical systems, while maintain both permeability and injectability. The liquid environment allows for a free-organization in the space of the cells towards the formation of new microtissues. The basic hybrid elements may be assembled towards larger constructs, using, for example, bioprinting methodologies, permitting to engineering tissues in a bottom-up approach with a complete control of the biological cargo.

Abstract **#65**

PROLIFERATION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS ON THERMORESPONSIVE SURFACE

Akon Higuchi[P][1,2,3], Hangze Ruan[3]

[1] *National Central University*

[2] *Riken*

[3] *Wenzhou Medical University*

Corresponding author's email: higuchi@ncu.edu.tw

Thermoresponsive surfaces enable the detachment of cells or cell sheets by decreasing the temperature of the surface when harvesting the cells. However, human pluripotent stem cells (hPSCs), such as embryonic stem cells and induced pluripotent stem cells, cannot be directly cultured on a thermoresponsive surface; hPSCs need a specific extracellular matrix to bind to the integrin receptors on their surfaces [1-3]. We prepared a thermoresponsive surface by using poly(N-isopropylacrylamide-co-butylarylate), recombinant vitronectin and/or laminin-511 to provide an optimal coating concentration for hPSC culture. In particular, we developed a repeated and continuous cultivation method of hPSCs on the same thermoresponsive plates where hPSCs were partially released from the same thermoresponsive plates by lowering the temperature of the thermoresponsive plates below the LCST of the thermoresponsive copolymer. Subsequently, the residual cells on the thermoresponsive plates were repeatedly and continuously cultivated in fresh cultivation media, and the detached stem cells were continuously collected. These cultivation cycles (passages) of hESCs as well as hiPSCs were successively repeated for ten cycles in this investigation. The detached cells, even after continual culture for over ten passages, showed high pluripotency, the ability to differentiate into cells derived from the 3 germ layers in vitro (Embryoid body formation assay) and in vivo (teratoma formation assay) as well as the ability to undergo cardiac differentiation.

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Abstract #66

FROM ORGAN-ON-A-CHIP TOOLS TOWARDS PATIENTS ON CHIPS – ENFORCING A PARADIGM SHIFT IN DRUG DEVELOPMENT

Uwe Marx[P][1]

[1] *TissUse GmbH, Germany*

Corresponding author's email: uwe.marx@tissuse.com

Microfluidic microphysiological systems (MPS) have proven to be a powerful tool for recreating human tissue- and organ-like functions at research level. This provides the basis for the establishment of qualified preclinical assays with improved predictive power. Industrial adoption of microphysiological systems and respective assays is progressing slowly due to their complexity. In the first part of the presentation status quo of MPS development and examples of industrial adoption of single-organ chip and two-organ chip solutions are highlighted. The underlying universal microfluidic Multi-Organ-Chip (MOC) platform of a size of a microscopic slide integrating an on-chip micro-pump and capable to interconnect different organ equivalents will be presented. Sixteen different single organ equivalents have been established on that platform and twelve organ combinations have been tested for stable long-term crosstalk yet. The second part of the presentation focusses on the challenges to translate a MOC-based combination of four human organ equivalents into a commercially useful tool for ADME profiling and toxicity testing of drug candidates. This four-organ tissue chip combines intestine, liver and kidney equivalents for adsorption, metabolism and excretion respectively. Furthermore, it provides an additional neuronal tissue culture compartment for extended toxicity testing. Issues to ensure long-term performance and industrial acceptance of such complex microphysiological systems, such as design criteria, tissue supply and on chip tissue homeostasis will be discussed. Finally, the presentation provides a roadmap towards on-chip patient models, which bear the potential of a paradigm shift in drug development.

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Abstract #67

IMMUNE MODULATION AS A NOVEL THERAPEUTIC CONCEPT IN REGENERATION - HARVESTING THE POTENTIAL OF T CELLS FOR BONE HEALING

Georg N. Duda[P][1,2], Christian H. Bucher[2], Claudia Schlundt[3], Hans-Dieter Volk[4], Katharina Schmidt-Bleek[3]

[1] *Julius Wolff Institut, Charité - Universitäsmedizin Berlin*

2 Berlin Institut of Health Centre for Regenerative Therapies

3 Julius Wolff Institut, Charité - Universitätsmedizin Berlin

4 Institut of Medical Immunology, Charité - Universitätsmedizin Berlin

Corresponding author's email: georg.duda@charite.de

Delayed or non-healing in bone is still a major clinical problem. Growing evidence shows that the specificity of inflammatory cells, the types of cytokines they secrete, and the timing of their appearance in the wound setting are essential for the healing cascade, revascularization and matrix formation. Understanding the local inflammatory response in healing is essential to control bone regeneration also in compromised settings.

We will present research showing that immune modulation can enhance bone regeneration. The complexity of the immune system and its numerous cell types offer several opportunities to employ immune modulation strategies: E.g. the induction of macrophage phenotype 2 over the inflammatory M1 phenotype or reduction of the number of pro-inflammatory CD8+ T cells in the wound bed. However, the optimal solution to stimulate healing by immune modulation is complicated to the tight interaction of the immune and skeletal system – influencing one without damaging the other.

Bringing basic understanding towards immune modulatory therapy is a challenge: Reducing negatively acting CD8+ effector T cells while simultaneously supporting beneficial regulatory T cells by raising the cAMP has been tested in vitro, in a pre-clinical experimental setting and will now be advanced towards a clinical trial. However, the immune composition changes with aging and differs individually. Thus, a careful stratification of patient is necessary in order to realize immune modulatory therapies. To realize translation of immune-modulatory strategies in regenerative medicine we need to broaden our basic understanding of the healing process and of the challenges that need to be overcome.

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Abstract #**68**

NEW ANIMAL MODELS TO INVESTIGATE MOLECULAR AND CELLULAR MECHANISMS OF CARDIOVASCULAR DISEASE AND PREMATURE AGING IN HUTCHINSON-GILDFORD PROGERIA SYNDROME

Vicente Andrés$_P$[1,2]

[1] *Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain*

2 CIBER de Enfermedades Cardiovasculares (CIBER-CV), Spain

Corresponding author's email: vandres@cnic.es

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by accelerated aging and death in adolescence mainly from cardiovascular disease (CVD). HGPS patients have a normal appearance at birth, and the disease is diagnosed during their first or second year of life after the development of clinical features. Most HGPS patients carry a heterozygous de novo synonymous point mutation in the LMNA gene (lamin A and C proteins). This mutation generates an aberrant LMNA splicing site that provokes the ubiquitous expression of progerin, a lamin A variant that induces multiple cellular alterations.

Mechanistic knowledge regarding how progerin accelerates CVD and aging has significantly increased in recent years. Using LmnaG609G knockin mice, we have identified mechanisms that contribute to vascular calcification, vessel stiffening, atherosclerotic disease, and cardiac repolarization defects in HGPS (1-6). We also demonstrated that restricting progerin expression to vascular smooth muscle cells (VSMCs) promotes endoplasmic reticulum stress/unfolded protein response, and is sufficient to accelerate vessel stiffness, contractile impairment and atherosclerosis, and reduces lifespan (2,3,7). We have generated new mouse models to address two questions that are relevant for translational and clinical HGPS research: 1) Can we prevent or slow down HGPS progression by ubiquitously suppressing progerin expression early after symptoms become apparent, and how late can we delay the initiation of treatment to achieve therapeutic benefit?; and 2) Can we prevent or slow down HGPS progression by suppressing progerin expression only in cardiovascular cells, major progerin targets? Some of our latest data will be presented.

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Abstract #69

STEM CELLS FOR ARTICULAR CARTIALGE ENGINEERING

Denis Evseenko[P][1]

[1] *University of Southern California*

Corresponding author's email: evseenko@usc.edu

Osteoarthritis (OA) impacts hundreds of millions of people worldwide, with those affected incurring significant physical and financial burdens. Injury to the articular surface is a major contributing risk factor for the development of OA. Current cartilage repair strategies are moderately effective at reducing pain but often replace damaged tissue with biomechanically inferior fibrocartilage. Here we describe the development, transcriptomic ontogenetic characterization and quality assessment at the single cell level, as well as the scaled manufacturing of an allogeneic pluripotent stem cell-derived articular chondrocyte formulation that exhibits long-term functional repair of porcine articular cartilage. We also provide some fundamental molecular differences between pluripotent stem cell and bone marrow stromal cell-derived chondrocytes. These results define a new potential clinical paradigm for articular cartilage repair and mitigation of the associated risk of OA.

Abstract **#70**

DEVELOPMENT AND CHARACTERISATION OF A NOVEL SYNTHETIC PEPTIDE BASED 3D MODEL OF PRIMARY OVARIAN CANCER – TOWARDS PERSONALISED THERAPEUTIC ASSESSMENT

Priyanka Gupta[P][1], Kavitha Madhuri Thumuluru[2], Aline Miller[3], Eirini Velliou[1]

[1] *University of Surrey, UK*

[2] *Royal Surrey County Hospital, UK*

[3] *Manchester BIOGEL, UK*

Corresponding author's email: priyanka.g.gupta@ucl.ac.uk

INTRODUCTION: Epithelial ovarian cancer (EOC) is the 8th most common cause of cancer related deaths in women [1], due to its late diagnosis, therapeutic resistance and high recurrence rate. The need for robust experimental models to help understand the EOC's pathology and metastasis have led to the development of 3D models in the form of spheroids and hydrogels [2,3]. Spheroids are high-throughput, easy to use but lack structural complexity and are difficult to maintain long term. Hydrogels add more complexity and are also capable of maintaining long term culture. Most currently available hydrogels for EOC use animal derived biomaterials (collagen, Matrigel) and lack the ability for growth surface and stiffness customisation. The aim of our work is to develop an animal free customised 3D model of EOC based on synthetic peptides with customised growth surface & stiffness.METHODS: EOC 3D cultures were established & maintained (4 weeks) using peptide-based hydrogels of different stiffness and charge. In vitro chemotherapeutic assessment was carried out within the established 3D models. Various in situ assays for monitoring the cell viability, spatial organisation and ECM production were carried out at specific time points. RESULTS: We successfully established a synthetic hydrogel-based 3D model of EOC wherein we observed that stiffness and charge of the hydrogels affected cellular growth and morphology. Furthermore, variability in terms of cellular viability and spatial organisation was found for different patients. Our model can be used as a rapid tool for personalised treatment screening of ovarian cancer.

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Abstract #71

CLINICAL EXPERIENCE OF TISSUE ENGINEERED VASCULAR GRAFTS IN CHILDREN

Toshiharu Shinoka[P][1]

[1] *Nationwide Children's Hospital*

Corresponding author's email: toshiharu.shinoka@nationwidechildrens.org

We have applied new protocol to FDA for the second generation of TEVG, including a rapid cell seeding technique, administration of Losartan, and higher dose of cell seeding in 24 patients as Fontan conduits. The rational design of improved, second-generation TEVGs will be predicated on our understanding of the cellular and molecular mechanisms underlying the formation of TEVG stenosis. The feasibility of tissue engineering large caliber, autologous vascular conduits for use as venous interposition grafts for congenital heart surgery has been demonstrated in both large animal and in a human clinical trial. The carefully designed second clinical trial in USA under the supervision of the FDA will be started in 2020.

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Abstract **#72**

GENERATION AND DIFFERENTIATION OF UNIVERSAL PLURIPOTENT STEM CELLS

Jhe-Yu Hsu$_P$[1], Akon Higuchi[1,2], Tzu-Cheng Sung[1], Yi-Peng Jiang[1]

[1] *Department of Chemical & Materials Eng., National Central University, Taoyuan, Taiwan*

[2] *Center for Emergent Matter Science, Riken, Saitama, Japan*

Corresponding author's email: hsujheyu1120@gmail.com

Millions of people suffer from loss and damage of organs and tissue every year due to accidents, birth defects, and disease. Human pluripotent stem cells (hPSCs) are a promising source of cells for tissue regeneration. However, histocompatibility of the transplanted cells remains a major challenge to their clinical application where human leukocyte antigen class (HLA) Ia (HLA-A, -B, -C) molecules are the primary mediators of immune reaction. We successfully generated universal induced pluripotent stem cells (hiPSCs) without gene editing, which were reprogrammed from human amniotic fluid stem cells with mixing two allogenic donors; these cells do not or less express human leukocyte antigen (HLA) class Ia (HLA-A, -B, and -C) and class II even after differentiation into cardiomyocytes, embryoid bodies (progenitor cells derived from three germ layers), and mesenchymal stem cells (universal hiPSCs). Cardiomyocytes differentiated from universal hiPSCs survived and continued beating even after treatment with allogenic mononuclear cells derived from different amniotic fluid (AF) donors. We speculate that the mechanism underlying the generation of universal hiPSCs is that fetal stem cells contain a specific group of cells that do not express HLA class Ia and class II but may express HLA-G, PD-L1 and/or CD47, which are expressed in baby tissue to avoid immune rejection with her mother, after the cells are reprogrammed into hiPSCs and subsequently differentiated into tissue cells.

Keywords

Universal stem cells; Pluripotency; Cell therapy

Abstract **#73**

WATERJET: A NOVEL TECHNOLOGY TO INJECT MYOBLASTS IN PREDETERMINED REGIONS OF THE URETHRAL SPHINCTER FOR IMPROVED CELL THERAPY OF URINARY INCONTINENCE

Ruizhi Geng[P1], Jasmin Knoll[1], Walter Linzenbold[2], Luise Jaeger[2], Markus Enderle[2], Tanja Abruzzese[1], Wilhelm K Aicher[1]

[1] *Dept. of Urology, UKT, Tuebingen, Germany*

[2] *ERBE Elektromedizin GmbH, Tuebingen, Germany*

Corresponding author's email: aicher@uni-tuebingen.de

Urinary incontinence is a significant medical challenge. Cell therapy may offer strategies to regenerate the sphincter function with high efficacy. Needle injection (NI) is the standard procedure but may misplace cells (1). We therefore developed a technology to inject cells by water jet (WJ) (2). We investigated if a) cells survive waterjet injections (WJI) with sufficient viability, b) the WJI inherits the risk of full tissue penetration of the urethra.

WJI were performed by a 2-phase injection as described (2). After WJI in capture fluid (n>5), cells were collected to determine the viability. For WJI in tissue, cells were labelled, injected (n>16), and either recovered and expanded in cultures or localized in cryosections by fluorescence microscopy.

WJI of myoblasts in capture fluid yielded mean viabilities of 88.3±3% or 87.9±2.1%. After injection in cadaveric samples followed by cell retrieval, differences in cell yield were not observed between WJI versus NI. However, NI in cadaveric samples analyzed in cryosections yielded more compact cell clusters compared to WJI. In WJI cells were distributed more precise and evenly. Injected cells presented with intact, round DAPI+ nuclei surrounded by Calcein+ cell somata indicating that myoblasts were intact after WJI.

We conclude that the novel WJT injects myoblasts precisely through the urothelial layer in the connective tissue of the urethra in a simple, fast, and precise way. Future studies will investigate the feasibility of the WJT in a living animal model and in a model of stress urinary incontinence (3).

Keywords

cell therapy; urinary incontinence; needle-feee cell injection technology

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Abstract #74

HARNESSING THE BIOMATERIAL-INFLAMMATORY CELL INTERACTIONS DURING THE DESIGN OF TISSUE ENGINEERED CONSTRUCTS

Donald O. Freytes[P][1,2]

[1] *Joint Department of Biomedical Engineering, NC State/UNC-CH, Raleigh, NC*

2 Comparative Medicine Institute, NC State, Raleigh, NC

Corresponding author's email: dofreyte@ncsu.edu

One of the goals of tissue engineering and biomaterial design is to create functional and viable tissue replacements or to design in vitro platforms using tissue surrogates to further understand tissue repair. One important process during tissue repair is the inflammatory response and the direct and indirect interactions between cells and the components of the engineered construct. Understanding these interactions and harnessing them to better understand and guide tissue repair is a fundamental goal that could lead to better integration and function of engineered constructs. Examples will be provided on how the interactions between biomaterials, repair cells and inflammatory cells can be used to guide the design, manufacture and preparation process. Inflammatory cells such as monocytes, macrophages and mast cells can be used to improve decellularization protocols, improve the survival of repair cells (i.e. cardiomyocytes, MSCs, etc) and to optimize extracellular matrix hydrogel preparations targeted to specific tissues such as heart, skin and vocal folds.

Abstract **#75**

DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO RETINAL PIGMENTED EPITHELIUM PROGENITORS ON ECM-COATED SURFACE

Yu Ru Zhu[P][1], Ming Wei Lu[1], Akon Highchi[1,2]

[1] *Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli,Taoyuan 32001, Taiwan*

[2] *Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama351-0198, Japan*

Corresponding author's email: lihibarike110391255@gmail.com

Introduction:

Age-related macular degeneration (AMD), according to world health organization, ranks the third leading cause of vision impairment and further causes irreversible blindness. This AMD disease mainly results from the dysfunction of retinal pigmented epithelium (RPE). Fortunately, transplanting human pluripotent stem cells (hPSCs)-derived RPE can serve as regenerative approach to cure AMD disease. However, RPE derived from hPSCs usually suffers from insufficient purity, long culture period and low yield. Therefore, we compared different protocols and cell culture extracellular matrices (ECMs) to investigate which conditions would be the most suitable for hPSCs to differentiate into RPE progenitors.

Method:

We first selected and compared the protocols, (i) N2 protocol [1] and (ii) NIC+CTM protocol [2]. After we found the better protocol, we tried different substrates to culture and induce differentiation of hPSCs into RPEs. We evaluated neural marker of PAX6 expression by using immunostaining and flow cytometry assay to find the best condition for hPSCs to differentiate in to RPE progenitors.

Results and Discussion:

According to epidermal marker of PAX6 analysis from immunostaining and flow cytometry assay, we found NIC+CTM protocol showed the better performance of hPSC differentiation into RPEs compared to N2 protocol. Subsequently, we found the optimal xeno-free culturing surface that could be applied for massive production of high purity of RPE progenitors in NIC+CTM protocol. We are planning to use the same protocol to further investigate the most appropriate substrate for differentiation into mature RPEs, which can benefit AMD patients for clinical treatment.

Keywords

Retinal Pigmented Epithelium; Human Pluripotent Stem Cells; Cell Therapy

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Abstract **#76**

3D IN VITRO MODEL OF THE PANCREATIC ACINO-DUCTAL UNIT THROUGH ADDITIVE MANUFACTURING TECHNOLOGY

Viola Sgarminato$_P$[1,2], Chiara Tonda-Turo[1,2], Gianluca Ciardelli[1,2,3]

[1] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy

[2] POLITO BIOMedLAB, Politecnico di Torino, Turin, Italy

[3] Department for Materials and Devices of the National Research Council, Institute for the Chemical and Physical Processes (CNR-IPCF UOS), Pisa, Italy

Corresponding author's email: viola.sgarminato@polito.it

The overall purpose of this work is to reproduce the morphology and composition of the pancreatic acino-ductal unit composed of specialized cells surrounded by stroma, a feature which is currently absent in 3D organoids modeling this functional unit1. This model serves as a powerful tool to investigate pathological processes such as pancreatic tumors (e.g. Pancreatic Ductal Adenocarcinoma, PDAC). The acino-ductal structure was reproduced through a 3D-bioprinting technology (ROKIT InVivo, Rokit, Seul) integrated with an atmospheric pressure plasma jet device (Stylus Plasma Noble, Nadir, Mestre). Specifically, the fabrication process was optimized to achieve high pore interconnectivity, accuracy in glandular geometry and precise control of pore size. 3D-printed structures morphology was characterized by optical and scanning electron microscopy (SEM). The images demonstrated the effectiveness of the optimization process which led to the formation of defect-free structures with interconnected pores. To introduce biomimetic cues within the polycaprolactone (PCL) structure, the plasma surface modification was implemented in a layer-by-layer and automatized manner in order to enhance the impregnation with a collagen solution. The collagen inside the pores allows cells to better colonize the structure and improves the adhesion, proliferation and growth of stromal cells inside the 3D structure as confirmed by morphological and cytochemical analysis. The cellularized final structure will provide a model of human PDAC at early stages which could be implemented in a microfluidic system. Advanced technologies which integrate optical components in microfluidic chips could be employed for the real-time monitoring of the tumor's evolution and for performing genomic analysis2.

Keywords

Additive manufacturing; 3D in vitro models; Pancreatic cancer

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Abstract **#77**

AN IN VITRO LUNG BIOMIMETIC MODEL

Michela Licciardello[P][1,2], Gianluca Ciardelli[1,2,3], Chiara Tonda-Turo[1,2]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *Polito BIOMedLab, Politecnico di Torino, Turin, Italy*

[3] *Department for Materials and Devices of the National Research Council, Institute for the Chemical and Physical Processes (CNR-IPCF UOS), Pisa, Italy*

Corresponding author's email: michela.licciardello@polito.it

The lung cancer, also known as lung carcinoma, is a malignant tumour characterized by uncontrolled cell growth in the specific tissue of respiratory system1. The lack of new therapies to treat lung carcinoma requires experimental models that can reproduce the behaviour of healthy and pathological tissues2. To overcome ethical, experimental and technological limitations of the traditional in vivo and in vitro models, we propose the implementation of a novel technological biomimetic platform to mimic the physiological condition by reproducing the basement membrane of alveolar wall and the physical stimuli characteristic of the blood-air barrier.

The basement membrane layer was reproduced through gelatin/polycaprolactone (GL/PCL) electrospun membrane obtained by mixing the two polymers (20:80 w/w) in a solution made of acetic and formic acid (1:1 v/v). A cold plasma treatment of the mats was carried out. The mechanical, physical and chemical properties of the mats were evaluated. A549 (lung epithelial cells, ATCC) and HULEC-5a (lung endothelial cells, ATCC) were cultured on the two side of electrospun membrane under static and dynamic conditions.

Scanning electron microscopy (SEM) images showed the nanofibers obtained after the electrospinning process. In vitro stability tests were performed to evaluate the degradation rate of the electrospun nanofibers. Cyclical traction tests were performed to assess the effect of the physiological breathing motion on membranes mechanical properties. The resazurin assay showed no significant reduction of cells viability after 7 days both in static and in dynamic condition.

The realized structures can be applied as ECM-mimic substrates for lung cells growth.

Keywords

Lung cancer; Electrospinning; In vitro tissue models

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Abstract **#78**

FABRICATION OF EXTRACELLULAR MATRIX -LIKE MEMBRANES FOR LOADING PIEZOELECTRIC NANOPARTICLES

Michela Licciardello$_P$[1,2], Chiara Tonda-Turo[1,2], Andrea Gallina[3], Gianni Ciofani[4], Gianluca Ciardelli[3,2,5]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy;*

[2] *Polito BIOMedLab, Politecnico di Torino, Turin, Italy*

[3] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[4] *Italian Institute of Technology (IIT), Pontedera (Pi), Italy*

[5] *Department for Materials and Devices of the National Research Council, Institute for the Chemical and Physical Processes (CNR-IPCF UOS), Pisa, Italy*

Corresponding author's email: michela.licciardello@polito.it

In the last two decades, piezoelectric nanomaterials have gained considerable attention in the biomedical community for their property to generate electrical activity in response to deformations and allow for the delivery of an electrical stimulus without the need of an external power source1. In tissue engineering applications, piezoelectric ceramic nanomaterial with perovskite-like structure have been widely combined with polymeric material, obtaining smart scaffold that promote the regeneration of electrically sensitive tissues such as bone 2 and nerve 3. In this work, we propose a method to fabricate extracellular matrix-like gelatin (GL) electrospun nanofibers with barium titanate nanoparticles (BTNPs)4.

Several GL (control) and GL/BTNPs solutions were prepared. Different concentrations of BTNPs were dispersed into the GL solutions (10% w/v to 30% w/v) and GA was added in order to improve the nanoparticles dispersion. The γ-glycidoxypropyltrimethoxysilane (GPTMS) agent was used to ensure the GL crosslinking. Nanofibrous mats were obtained after optimizing the electrospinning process parameters.

Scanning electron microscopy (SEM) images showed randomly-oriented nanofibers where BTNPs appeared uniformly distributed over the length of the composite fibres. Thermogravimetric analysis (TGA) confirmed the encapsulation of the nanoparticles into the fibres. The presence of BTNPs did not influence the viability of SaOS-2 cells. Therefore, an increase of calcium deposition was observed in MSCs cultured on piezoelectric substrate.

Electrospun composite nanofibers were successfully obtained and characterized demonstrating a strong potential as ECM-mimic substrates for tissue engineering application.

Keywords

Piezoelectric nanomaterials; Electrospinning; Tissue engineering

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Abstract **#79**

ESTABLISHMENT OF PATIENT-SPECIFIC CANCER CELL LINES BY MEMBRANE FILTRATION METHOD VIA NYLON MESH FILTER AND PLGA-SILK SCREEN MEMBRANES

JIA HUA WANGP[1], WEI LUN HUANG[1], YI PENG JIANG[1], Akon Higuchi[2]

[1] *Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli, Taoyuan 32001, Taiwan*

[2] *Riken Cluster for Pioneering Research, RIKEN, Wako, Saitama 351-0198, Japan*

Corresponding author's email: a0922416954@gmail.com

Cancer is one of the top 10 leading cause of death in world. Cancer stem cells (CSCs) are responsible for cancer proliferation and show stronger resistance than other cancer cells under conventional cancer therapy. Recent publication suggested the precision medicine shows positive effect for cancer patients treatment so isolation of patient specific primary cancer cell line is important. However, it is hard to distinguish CSCs from other cells in primary tissue. Thus, we have been developing a membrane filtration method to target CSCs.

This membrane filtration method was performed via Nylon mesh filter or poly(lactic-co-glycolic acid)-silk screen membranes. The primary colon cancer cells were isolated by several steps and the cancer cell solution was permeated through these membranes. CSCs and colon cancer cells were characterized using colony forming assay and expression of carcinoembryonic antigen (CEA) using ELISA assay. We also analyzed colon cancer cell lines using this method as a model of primary colon cancer tissue solution.

This purification method using membrane filtration method has been successfully verified using Lovo cancer cell line and the cells showing high level expression of CSCs marker (CD133, CD44) were isolated where CSC markers were analyzed using flow cytometry. We expect to establish patient specific colon cancer cell line by the membrane filtration method with different pore size, affinity and biocompatibility. This method is a promising approach, which can be applied for precision medicine screening in the future.

Keywords

cancer stem cells; membrane

Abstract **#80**

DETECTING EARLY OSTEOARTHRITIS PATHOLOGY THROUGH QUANTITATIVE AI-SUPPORTED OPTICAL BIOPSY USING CLINICALLY AVAILABLE TECHNOLOGY

Mischa Selig[P][1]

[1] *G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Dept. of Orthopedics and Trauma Surgery; Albert-Ludwigs-University of Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany*

Corresponding author's email: mischa.selig@uniklinik-freiburg.de

INTRODUCTION: Clinical trials are unable to pinpoint early onset of osteoarthritis (OA). Thus, we propose the superficial zone chondrocyte spatial organization (SCSO) for visualization and quantitative input for predictive modelling and user-independent diagnosis.

METHODS: Discs from human OA articular cartilage (AC) were fluoro-tagged and imaged with a fluorescence microscope and clinically available endomicroscope. Each SCSO was classified as chondrocyte string, double string, small cluster, big cluster, or diffuse organization. Multiple quantitative SCSO parameters were calculated for each disc through point pattern analysis and used for training a random forest model (RF).

RESULTS: We visualized all SCSO stages and quantified each stage using established spatial analysis parameters like the nearest neighbor cell-cell-distances (NNDs), cell intensity, the Clark-Evans-Aggregation-Index, the pair correlation function, and others. These were used for RF modelling. 50 different RFs were evaluated based on randomly sampling the input data. 22% of RFs classified the SCSO accurately in more than 90% of cases, and one model classified the SCSO accurately in 100% of cases with an excellent RF multiclass model performance measured via Matthews correlation coefficient (0.9229) and high reproducibility (100%). This selected RF model also demonstrated 100% accuracy in determining the SCSO stage of endomicroscopy-recorded SCSO stages.

DISCUSSION & CONCLUSIONS: We demonstrated that the SCSO, an experimental marker for early OA, can be visualized with currently available clinical technology. Moreover, quantitative data derived from the SCSO was suitable to train a RF model with 100% accuracy for correctly diagnosing early changes in SCSO, and thus early OA.

Abstract **#81**

HYDROSTATIC PRESSURE CAN INDUCE APOPTOSIS OF THE SKIN

Tien Minh Le$_P$[1,2], Naoki Morimoto[3], Nhung Thi My Ly[4], Toshihito Mitsui[2], Sharon Claudia Notodihardjo[2], Natsuko Kakudo[2], Kenji Kusumoto[2]

[1] *Department of Orthopedics and Traumatology, SAIGON ITO Hospital, Ho Chi Minh City, Vietnam*

[2] *Department of Plastic and Reconstructive Surgery, Kansai Medical University, Hirakata, Osaka, Japan*

[3] *Department of Plastic and Reconstructive Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan*

[4] *Department of Dermatology, Kansai Medical University, Hirakata, Osaka, Japan*

Corresponding author's email: drminhtienle@gmail.com

We previously showed that high hydrostatic pressure (HHP) treatment at 200 MPa for 10 min induced complete cell death in skin and skin tumors via necrosis. We used this technique to treat a giant congenital melanocytic nevus and reused the inactivated nevus tissue as a dermis autograft. However, skin inactivated by HHP promoted inflammation in a preclinical study using a porcine model. Therefore, in the present study, we explored the pressurization conditions that induce apoptosis of the skin, as apoptotic cells are not believed to promote inflammation, so the engraftment of inactivated skin should be improved. Using a human dermal fibroblast cell line in suspension culture, we found that HHP at 50 MPa for ≥36 h completely induced fibroblast cell death via apoptosis based on the morphological changes in transmission electron microscopy, reactive oxygen species elevation, caspase activation and phosphatidylserine membrane translocation. Furthermore, immunohistochemistry with terminal deoxynucleotidyl transferase dUTP nick-end labeling and cleaved caspase-3 showed most cells in the skin inactivated by pressurization to be apoptotic. Consequently, in vivo grafting of apoptosis-induced inactivated skin resulted in successful engraftment and greater dermal cellular density and macrophage infiltration than our existing method. Our finding supports an alternative approach to hydrostatic pressure application.

Abstract **#83**

IL-17 CONTROL OF CHONDROCYTE NUCLEAR AND CELL SHAPE AND FUNCTION: POSSIBLE IMPACT ON CARTILAGE REPAIR MECHANISMS

Melanie L. Hart$_P$[1], Judith M. Horvath[1], Anna De Giuseppe[1], Eunjae Park[1], Ronja Sturm[1], Mischa Selig[1], Jasmin C. Lauer[1], Marita Völker[1], Bernd Rolauffs[1]

[1] *G.E.R.N. Center for Tissue Replacement, Regeneration & Neogenesis, Dept.of Orthopedics & Trauma Surgery, Medical Center-Albert-Ludwigs University of Freiburg, Germany*

Corresponding author's email: melaniehar@gmail.com

IL-17 has been implicated in cartilage degradation and inflammation. We aimed to understand how inflammation, cell morphology and cell function are linked. Articular cartilage superficial zone explants and chondrocytes from patients having different grades of macroscopic osteoarthritic (OA) degeneration were examined to determine a) whether there was a differential response of isolated chondrocytes to IL-1β vs. IL-17A and b) if OA cartilage explants responded similarly. Explants were exposed to injury and inflammatory cytokines, while chondrocytes were exposed to inflammatory cytokines. IL-1β caused a significant decrease in COL2A1 gene expression in both tissue explants and chondrocytes. IL-17A also resulted in a significant decrease in chondrocyte COL2A1 expression. In tissue explants, COL2A1 showed a decreasing trend in response to IL-17 and the decrease in expression was similar to IL-1β treatment. In contrast, in comparison to IL-1β, IL-17A resulted in a significantly decreased inflammatory response (IL-8 gene expression) in both chondrocytes (59-fold decrease, 24h; 10-fold decrease, day 7) and OA explants (32-fold decrease, 48h). Despite a decreased IL-8 mRNA expression response, IL-17A caused high secretion of soluble IL-8 from chondrocytes which correlated dose-dependently with nuclear size and shape. In chondrocytes, COL1A2 expression was not significantly altered by IL-17A, but its expression significantly correlated with cell aspect ratio, roundness, circularity, and solidity and nuclear aspect ratio and roundness. Importantly, IL-17A dose-dependently decreased COL2A1 expression which correlated with chondrocyte roundness and nuclear aspect ratio and roundness. Therefore, IL-17A is capable of controlling cell and nuclear shape and chondrogenic cell function which may impact cartilage repair.

Keywords

Inflammation; Cell shape; IL-17

Abstract **#84**

3D MODELLING STRATEGIES TO PREDICT THE MECHANICAL BEHAVIOUR OF POLYMERIC SCAFFOLDS BY ADDITIVE MANUFACTURING

Mario Monzón[1], Gisela Vega[1], Rubén Paz[1], Andy Gledall[2], María E. Alemán[1]

[1] *Department of Mechanical Engineering. University of Las Palmas de Gran Canaria. Spain*

[2] *Wolfson School of Mechanical and Manufacturing Engineering, UK Loughborough University*

Corresponding author's email: mario.monzon@ulpgc.es

Additive Manufacturing (AM) is a well known method to produce scaffolds with different geometrical patterns (grid, honeycomb, gyroid, etc.). These hierarchical porous structures can be designed ad hoc to enable specific mechanical properties and functionalities to provide cell viability and tissue regeneration. In particular, the AM technology based on material extrusion (MEX, commonly known as FDM) produces 3D porous structures processing several thermoplastic biopolymers. Nevertheless, the operational parameters and the characteristics of the deposition process generate a part in which real geometry is different from the theoretical one of the 3D model. This difference involves an additional problem for simulating the mechanical behaviour of the scaffold, for example by Finite Element Analysis (FEA). The paper presents two developed methods to predict the real geometry of scaffolds made by MEX and starting from G-code files. The first method is the "automated sweep CAD modeller of extrusion-based G-code (DECODE)" and the second the "volume conserving model for 3D printing (VOLCO)". DECODE reads the G-code and generates several scripts to automate the 3D CAD modelling (sweep features) to reproduce the extrusion paths. VOLCO builds a voxel-based model starting from the G-code and considering some features in the deposition process. Both methods were compared in terms of volume of the resulting geometry of the scaffolds, and the FEA simulation and testing of the stiffness. DECODE obtains more accuracy in volume (compared to the theoretical models) and can manage larger parts, while VOLCO, after a fitting process, is more accurate for mechanical prediction by FEM.

Abstract #85

SOLUTIONS FOR REPOPULATION OF DENSE CARTILAGE MATRIXES.

Sylvia Nürnberger[P][1,2,3]

1 Medical University of Vienna, Department of Orthopedics and Trauma-Surgery, Division of Trauma-Surgery

2 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Trauma Research Center

3 Austrian Cluster for Tissue Regeneration

Corresponding author's email: sylvia.nuernberger@meduniwien.ac.at

Decellularised organs and tissues are promising scaffolds for tissue regeneration providing the accurate composition and architecture to support targeted tissue regeneration. In this process, repopulation of devitalized matrixes is the most critical and challenging step, especially in dense tissues such as cartilage. To overcome this difficulty, several chemical and mechanical strategies have been developed. Auricular cartilage can be enzymatically extracted from specific matrix components such as elastin, creating channels accessible for cells to grow into the dense matrix. However, for articular cartilage chemical treatment for selective removal of matrix components is not sufficient to achieve repopulation. As alternative, laser perforation has been developed allowing to engrave fine incisions of controlled and reproducible size by a high throughput process. Two of the most commonly used laser technologies used in the medical field, the CO2 and femtosecond laser, were applied to hyaline cartilage with very different structural effect. Within this talk, the structuralizing possibilities of laser and enzymatic treatments, the effect on the matrix and the general advantages and disadvantages for tissue engineering are discussed. We believe that the optimal combination of chemical and laser treatment have high potential for a new generation of biomaterials for tissue engineering.

Abstract **#86**

ADIPOSE TISSUE-DERIVED STROMAL CELLS ALTER HYDROGEL VISCOELASTIC PROPERTIES BEFORE PATIENT IMPLANTATION

Francisco D Martinez Garcia[P][1], Martine M Valk[1], Prashant K Sharma[1], Janette K Burgess[1], Martin C Harmsen[1]

[1] *University Medical Center Groningen*

Corresponding author's email: f.d.martinez.garcia@rug.nl

Gelatine methacryloyl (GelMA) is a highly tunable, inexpensive hydrogel that can be loaded with adipose tissue-derived stromal cells (ASC) for therapeutic purposes. Information on the ASC remodelling of GelMA and its influence on mechanical properties is scarce. Yet, changes potentially affect the fate and function of GelMA-ASC constructs before patient implantation. Our study assessed if ASC modified the stiffness and viscoelasticity of GelMA hydrogels aimed for tissue engineering. Methods: Immortalised human ASC (iADSC13) were embedded in 5%, 10% and 15% (w/v) GelMA hydrogels. Cell-loaded and cell-free (control) hydrogels were photopolymerised. Viscoelastic changes in stiffness and the percentage of stress relaxation were measured by low-load compression testing at 20% strain s-1. Live and dead fluorescent staining assessed cell viability. Evaluation occurred at 0 h, 24 h, 7 d and 14 d. Results: GelMA hydrogels had a linear concentration-dependent increase in stiffness while cells caused a time-dependent decrease. In 5% GelMA, cells increased the percentage of stress relaxation, in 15% GelMA it decreased while in 10% GelMA decreased occurred only at 7 d. Initial cell viability was >90%, which prevailed in 5% GelMA over 14 d, while in 10% and 15% viability decreased. Conclusions: The data shows that the viscoelastic properties of GelMA hydrogels change due to cell-matrix interactions over time, but that stress relaxation changes occur independently of changes in the stiffness.

Abstract #88

4D PATTERNING OF CELL-LADEN BIOMATERIALS THROUGH MULTIPHOTON LITHOGRAPHY

Cole A. DeForest[P][1,2,3]

1 Department of Chemical Engineering, University of Washington

2 Department of Bioengineering, University of Washington

3 Institute for Stem Cell and Regenerative Medicine, University of Washington

Corresponding author's email: profcole@uw.edu

The extracellular matrix directs cell function through a complex choreography of biomacromolecular interactions in a tissue-dependent manner. Far from static, this hierarchical milieu of biochemical and biophysical cues presented within the native cellular niche is both spatially complex and ever changing. As these pericellular reconfigurations are vital for tissue morphogenesis, disease regulation, and healing, in vitro culture platforms that recapitulate such dynamic environmental phenomena would be invaluable for fundamental studies in cell biology, as well as in the eventual engineering of functional human tissue. In this talk, I will discuss some of our group's recent success in reversibly modifying both the chemical and physical aspects of synthetic cell culture platforms with user-defined spatiotemporal control. Innovating biomaterials sensitive to cytocompatible light, we exploit multiphoton laser-scanning lithographic strategies to create and photochemically modify biomaterials around living cells. Repurposing tools traditionally employed for biological imaging to directly manipulate biomaterials, we have gained the unique ability to modulate intricate cellular behavior including stem cell differentiation, protein secretion, and cell-cell interactions in 3D space and time (i.e., 4D).

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Abstract **#89**

DECELLULARIZED PORCINE NUCLEUS PULPOSUS TISSUE FOR INTERVERTEBRAL DISC REGENERATION

Tara C Schmitz$_P$[1], Marina van Doeselaar[1], Keita Ito[1]

[1] *Orthopaedic Biomechanics, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands*

Corresponding author's email: t.c.schmitz@tue.nl

Tissue-derived decellularized scaffolds are increasingly employed in tissue engineering for regenerative medicine. During decellularization cells within the extracellular matrix (ECM) are lysed using detergents/freeze-thaw cycles, frequently depleting essential matrix components like growth factors, collagen and glycosaminoglycans (GAGs).1–3 Porcine notochordal cell-derived matrix (NCM) has gained attention due to its beneficial effect on degenerated intervertebral discs. Notochordal cells secrete a set of soluble stimulating factors leading to increased ECM production by resident intervertebral disc cells.4–7 For its use as a therapeutic agent in human patients, porcine DNA must be removed from NCM to ensure safety regarding possible transmission of porcine endogenous retroviruses (PERVs) to the patient.8, 9 Here, we present a simple, detergent-free protocol for DNA digestion within NCM while maintaining its bioactive features. Cell lysis is achieved via tissue lyophilization, and >90% of porcine DNA is digested with high amounts of endonuclease while tissue dissociation is prevented by the low buffer volume used. No significant loss in GAG and collagen content was found, while >40% proteins remain. Endonuclease-remnants do not impair human fibroblast cell viability. Residual bioactivity is demonstrated by incubation of bovine NP cells with the decellularized NCM compared to non-processed NCM. We find that bioactivity is reduced after tissue processing, but still promotes NP cell-typical gene expression and matrix deposition, and decreases the inflammatory response. Further encapsulation of this decellularized NCM into a NP-like biomaterial may constitute a future regenerative treatment for degenerative disc disease providing biomechanical restoration of the disc in addition to its regenerative effect.

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Abstract **#90**

WIN, LOSE OR TIE: A COMPUTATIONAL MODEL OF THE COMPETITION AT THE CELL-ECM INTERFACE

Zeynep Z Karagöz[1], Thomas Geuens[1], Vanessa L.S. LaPointe[1], Martijn van Griensven[1], Aurélie Carlier[1]

[1] *The Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine*

Corresponding author's email: z.karagoz@maastrichtuniversity.nl

The extracellular matrix (ECM) is a mesh of fibrous proteins that forms the basis of the tissue architecture and structurally supports the cells. Biophysical cues provided by the ECM are translated into biochemical signals by transmembrane integrin molecules. Understanding the interaction between the ECM and integrin molecules is particularly important when designing instructive biomaterials and organoid culture systems. Previous studies suggest that fine-tuning the ECM composition and mechanical properties can improve organoid development [1]–[3]. Towards the bigger goal of fully functional organoid development, we hypothesize that resolving the dynamics of ECM-integrin interactions will be highly instructive. To this end, we developed a mathematical model that enabled us to simulate three main interactions, namely integrin activation, ligand binding and integrin clustering. Different from previously published computational models, we account for the binding of more than one type of ligand to the same type of integrin. This competition between ligands defines the fate of the system. We have demonstrated that the ligand with higher binding rate occupies more integrins at the steady state than does the competing ligand. We have also demonstrated that an increase in the initial concentration of ligands does not ensure an increase in the steady state concentration of ligand-bound integrins. With cell type specific, quantitative input on integrin-ligand binding rates, this model can be used to develop instructive cell culture systems.

Keywords

integrin; extracellular matrix; reaction kinetics

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Abstract #91

KIDNEY-ON-A-CHIP PLATFORMS FOR DISEASE MODELING AND DRUG TESTING

Roos Masereeuw[P][1]

[1] *Utrecht Institute for Pharmaceutical Sciences, div. Pharmacology*

Corresponding author's email: r.masereeuw@uu.nl

The kidneys play an essential role in preserving homeostasis of the body's internal environment, including regulation of water and acid-base balances, waste removal, control of red blood cell production and blood pressure regulation. Impaired renal function is commonly observed in clinical practice and is often associated with use of drugs. Early prediction of those effects, which includes drug-drug interactions and renal toxicity, is crucial for the development of new and safe drugs by the pharmaceutical industry. Currently available in vitro assays do not accurately allow such prediction, predominantly due to inadequate preservation of the organs' microenvironment. The kidney epithelium is highly polarized, and the maintenance of this polarity is critical for optimal functioning and responsiveness to environmental signals influencing cell proliferation, migration and differentiation. This presentation will provide an overview of advances in 3D cultures of human kidney cells and organoids in microfluidics, and in particular kidney tubules, thereby improving physiological performance of the tissue. These kidney-on-a-chip platforms have great potential for disease modeling and drug screening, and provide novel alternative strategies for prediction of renal drug disposition and safety assessment in a human-specific context.

Abstract **#92**

BEATING ORGANS-ON-CHIPS AS ADVANCED PRECLINICAL TOOLS FOR DRUG SCREENING AND DISEASE MODELLING APPLICATIONS

Paola Occhetta[P][1,2], Roberta Visone[1,2], Andrea Mainardi[2,3], Andrea Barbero[3], Marco Rasponi[2]

[1] *BiomimX Srl, Milano, Italy*

[2] *MiMic Lab, Department of Electronics, Informatics and Bioengineering, Politecnico di Milano, Milano, Italy*

[3] *Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland*

Corresponding author's email: paola.occhetta@polimi.it

Organs-on-Chip (OoC) have recently emerged as innovative in vitro tools holding the potential to improve prediction over human drug responses. Here we present new beating OoC, advanced platforms integrating native-like 3D mechanical microenvironment with an unprecedented level of precision. This is achieved through uBeat®, an innovative technology(1) that allows to modulate mechanical deformation exerted on 3D microtissues in a controlled fashion. Two uBeat®-based models were developed: uHeart, a beating heart-on-chip integrating real-time electrophysiological measurements, and uKnee, the first in vitro model of human osteoarthritic (OA) cartilage-on-chip.

uHeart provides 3D human cardiac microtissues with a physiological cyclic uniaxial strain (10%, 1Hz). Cardiomyocytes from human induced pluripotent stem cells and human fibroblast embedded in fibrin hydrogel and cultured within uHeart developed in synchronously beating cardiac microtissues(2), as confirmed by on-chip electrophysiology studies. uKnee provides 3D cartilage-on-chip (CoC) with hyper-physiological (HP) compression (30%, 1 Hz), sufficient to elicit OA pathogenesis in vitro. Healthy CoC were generated from human articular chondrocytes embedded in poly(ethylene-glycol)-based hydrogel. HP stimulated CoC showed a shift in cartilage homeostasis towards catabolism, inflammation and hypertrophy, and acquisition of a gene profile compatible with OA clinical evidences(3). Both models were successfully exploited for testing the effect of well-known drugs and innovative compounds.

Integration of 3D mechanical microenvironment resulted in OoC models with enhanced functionality and resemblance to pathological states. uBeat® is highly versatile and applicable to any organ in which mechanical stimulation exerts a pathophysiological state. Beating OoC represent new powerful tools for in vitro drug screening and disease modelling.

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Abstract **#93**

3D BIOMATERIAL BASED REMODELLING OF HYPOXIA FOR RADIOTHERAPY SCREENING OF PANCREATIC CANCER

Gabrielle Wishart$_P$[1], Priyanka Gupta[1], Giuseppe Schettino[1,2], Andrew Nisbet[3], Eirini Velliou[1]

[1] *University of Surrey, UK*

[2] *National Physical Laboratory, UK*

[3] *University College London, UK*

Corresponding author's email: e.velliou@surrey.ac.uk

INTRODUCTION

Tissue engineering to mimic in vivo milieus is fast advancing to support representative biophysical-chemical and mechanical tumour properties allowing realistic architecture, cell-matrix interactions and environmental gradients. Pancreatic ductal adenocarcinoma (PDAC) is a cancer of unmet needs with a notoriously unique and radio-resistant tumour microenvironment (TME). 3D re-modelling of the PDAC TME for radiation treatment screening is an understudied area of research. The BioProChem Group at the University of Surrey have previously fabricated a 3D porous polymeric scaffolding system to support long term PDAC cell growth and in vivo properties [1-3]. Utilising this system, we aim to replicate the PDAC TME incorporating long term hypoxic exposure to investigate hypoxia induced radio-resistance for the clinical application and optimisation of radiotherapy.

METHODS

Fabrication of polymeric 3D scaffolds employed the Thermally Induced Phase Separation method [1]. Scaffolds were coated in the extracellular matric protein fibronectin to aid cellular adhesion. PANC-1 cells were seeded at 0.5x106 and cultured for 3 weeks before being placed at 5% oxygen in a hypoxic chamber. Radiotherapy exposures were performed using an orthovoltage X-ray unit at the Royal Surrey County Hospital. Scanning electron microscopy and confocal laser Scanning microscopy (CLSM) enabled scaffold characterization, allowing analysis of cellular organisation and viability. More specifically, live/dead analysis and hypoxic biomarkers permit quantification of radio-resistance.

CONCLUSIONS

For the first time, this research develops a hypoxic PDAC 3D polymeric scaffold to support long-term cell culture. This platform allows hypoxia-associated radio-resistance profiling of PDAC towards radiation response screening for a cancer with such dismal prospects.

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Abstract **#94**

TOWARDS A BIOMIMETIC LOAD DISTRIBUTION IN TISSUE ENGINEERED HEART VALVES: A NOVEL APPROACH

Christian Boehm$_P$[1], Stefan Jockenhoevel[1,2], Maximilian Werner[1], Lukas Lamm[3], Stafanie Reese[3], Timothee Noterdaeme[4]

[1] *AME BioTex – Biohybrid & Medical Textiles, RWTH Aachen University, Aachen, Germany*

[2] *Aachen-Maastricht Institute for Biobased, Sciences, Faculty of Science and Engineering, Maastricht, Netherlands*

[3] *Institut für Angewandte Mechanik RWTH Aachen University, Aachen, Germany*

[4] *Departement of Internal medicine I, RWTH Aachen University Hospital, Aachen, Germany*

Corresponding author's email: boehm@ame.rwth-aachen.de

The challenge of Tissue Engineering hydrogel contraction during tissue maturation leads to a changed 3D geometry and thus dysfunction of the target tissue. To minimize contraction and enable biomimetic loadbearing of the engineered tissue we want to develop a textile-reinforced structure tailored to specific needs of an aortic valve.

In order to analyze tissue contraction we established a novel method based on the work by Truskey et. al to evaluate tissue contraction in static and dynamic cultivation. We performed multiple assays to evaluate tissue contraction and composition. Based on the results we developed a novel load specific textile scaffold for a tissue engineered aortic heart valve. In combination with a novel stent, we were able to measure good hemodynamics with a silicone heart valve under aortic conditions.

The hemodynamic tests showed results with in the ISO 8540 standard (a Total regurgitant fraction <20 % and an effective orifice area > 1.25mm² for a 23mm Valve). Preliminary tests in a low-pressure environment of a cell laden reinforced fibrin valve show good collagen production and cell mediated shrinkage is in an acceptable range.

Our biomimetic load specific scaffold can improve the in vitro results of heart valve conditioning in a low-pressure environment. The next step is to repeat the experiments in aortic conditions. This will be a major step towards a tissue engineered aortic heart valve.

Abstract **#95**

EFFECTS OF SPACEFLIGHT ON HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE STRUCTURE AND FUNCTION

Arun Sharma[P][1]

[1] *Cedars-Sinai Medical Center*

Corresponding author's email: arun.sharma@cshs.org

With extended stays aboard the International Space Station (ISS) becoming commonplace, there is a need to better understand the effects of microgravity on cardiac function. We utilized human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to study the effects of microgravity on cell-level cardiac function and gene expression. The hiPSC-CMs were cultured aboard the ISS for 5.5 weeks and their gene expression, structure, and functions were compared to ground control hiPSC-CMs. Exposure to microgravity on the ISS caused alterations in hiPSC-CM calcium handling. RNA-sequencing analysis demonstrated 2,635 genes were differentially expressed among flight, post-flight, and ground control samples, including genes involved in mitochondrial metabolism. This study represents the first use of hiPSCs to model the effects of spaceflight on human cardiomyocyte structure and function.

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Abstract **#98**

THE OSTEOCONDUCTIVITY OF THE AMORPHOUS AND CRYSTALLINE COATINGS IN THE ABSENCE AND PRESENCE OF INCORPORATED BOVINE SERUM ALBUMIN (BSA) IN VITRO AND IN VIVO

Menghong Li[1,2], Gang Wu[1,2], Yuelian Liu[1,2]

[1] *Academic Centre for Dentistry Amsterdam(ACTA)*

[2] *University of Amsterdam and Vrije Universiteit Amsterdam*

Corresponding author's email: m.li@acta.nl

Background and Aim:Biomimetic calcium phosphate (CaP) coatings have been developed for coating medical device as a slow and local drug delivery system.The aim of this study was to compare the osteoconductivity of the amorphous and crystalline coatings in the absence and presence of incorporated bovine serum albumin (BSA) in vitro and in vivo. Material and methods:Amorphous and crystalline coatings were prepared. Deposition and chemical property were examined by LSCM and FTIR respectively.Surfaces of implants were characterized by SEM.Loading and release of BSA were evaluated by ELISA. The activity of ALP were tested in vitro.6 different groups were used in vivo:no coating no BSA, no coating but with surface adsorption of BSA and incorporation of BSA in the biomimetic coating in the amorphous and crystalline coatings.Rats orthotopic model was used (n=6 rats per group).Time points are 3 days,1,2 and 4 weeks.Histological and histomorphormitrical ananlysis were done in order to determine bone to implant contact(BIC).Results:In vitro,the protein the morphology of BSA incorporated crystalline coating was changed into curly plates, and it shows slow-release profile.The BSA incorporated crystalline coating significantly decreased the activity of ALP.In vivo study, each group showed time-dependent increase in BIC.The incorporation of BSA had no significant effect on the percentage of BIC in no coating groups and crystalline coating groups,but resulted in lower BIC in amorphous coating groups.Conclusion: Biomimetic crystalline CaP coating can be an ideal carrier for protein or drugs;the amorphous biomimetic CaP coating promoted osteoconductivity and incorporation of BSA did not improve osteogenesis.

Keywords

calcium phosphate; biomimetic coating; osteoconductivity

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Abstract **#99**

BIOFABRICATION OF A VASCULAR-LIKE STRUCTURE MIMICKING THE ARCHITECTURE AND CELL ALIGNMENT OF NATIVE BLOOD VESSELS

Elia Bosch-Rué[1], LM Delgado[1], Leire Díez-Tercero[1], Raquel Rodríguez-González[1], BM Bosch-Canals[1], FJ Gil[1], Román A Pérez[1]
[1] *Bioengineering Institute of Technology (BIT), Universitat Internacional de Catalunya (UIC), Barcelona, Spain*
Corresponding author's email: ebosch@uic.es

Cardiovascular diseases (CVD) are the main cause of worldwide death according to WHO (1), being the obstruction of vessels due to atherosclerosis the principal etiology. Up to now, the gold standard treatment has been blood vessel replacement from autologous source through bypass surgery. However, this is not always possible due to the inherent disease of the patient or previous harvest. Consequently, tissue engineered blood vessels (TEBV) have been considered as a promising alternative as a source graft (2). The aim of this study was to develop a mechanically stable dual-layered hydrogel hollowed structure where specific vascular cell types could be allocated, mimicking the microarchitecture of native blood vessels. Using a triple co-axial extrusion system, outer and inner layers were obtained, mainly composed of alginate and collagen, and hollow core was achieved using a sacrificial polymer. The TEBV presented homogeneous sizes, which could be controlled by changing the injection speed, and they were robust, allowing the perfusion of liquids. Human umbilical vein endothelial cells (HUVECs) and human aortic smooth muscle cells (HASMCs) were encapsulated in the inner and outer layer, respectively, presenting high survival rates (over 90%) after extrusion and after 20 days of culture. Moreover, both cell types could proliferate within the TEBV. Interestingly, HUVEC and HASMC could adopt the native alignment found in native vessels, being the concentric arrangement of HASMCs of special interest, as it facilitates the vasoconstriction. Further studies involving perfusion of these TEBV-like structures are required in order to induce maturation and a proper functionality.

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Abstract **#100**

COMPARISON OF NOTOCHORDAL CELL MARKERS EXPRESSION BETWEEN 2D AND 3D IN VITRO CULTURE SYSTEMS

Shaghayegh Basatvat$_P$[1], Rebecca Williams[1], Abbey Binch[1], Lisanne Laagland[2], Adel Medzikovic[2], Frances C Batch[2], Deepani Poramba Liyanage[2], Keita Ito[3], Marianna A Tryfonidou[2], Christine Le Maitre[1]

[1] *Biomolecular Science Research Centre Department of Bioscience and Chemistry, Sheffield Hallam University, Sheffield, UK*

[2] *RMCU, Utrecht University, Netherland*

[3] *Institute for Complex Molecular Systems, TU/e Institute, Netherland*

Corresponding author's email: s.basatvat@shu.ac.uk

Intervertebral disc (IVD) degeneration accompanying with lower back pain is a serious worldwide problem. Although, surgical treatments are available for pain relief, we need to establish enduring cell-based remedies. Notochordal (NC) cells, characterized by large vacuoles, as the ancestor of nucleus pulposus (NP) cells in human IVD, are a promising therapeutic target. Thus, we aimed to optimise the culture of NC cells in vitro without using Fetal Calf Serum (FCS) and investigate their morphology and gene expression in both 2D and 3D culture systems.

Porcine NC cells were extracted using pronase treatment followed by overnight collagenase II digestion. Extracted cells were culture in either 1.2% alginate beads (3D culture) with 21% or 5% oxygen or laminin-coated plates (2D culture) with 5% oxygen. Cells were harvested after 24 hours, 1 and 2 weeks for gene expression and immuno-staining analysis of NC and NP markers (CD24, KRT8, KRT18, KRT19, ACAN, COLII, PAX1, FOX-F1 and T).

A mixed phenotype of NC and NP cells was observed in alginate bead cultures. NCs were observed within all culture conditions with production of GAGs and maintenance of the vacuolated phenotype. In 21% O2 culture, a rim effect was observed with more NC cells toward the edges of the beads while in hypoxia, although a rim effect was observed, more NCs were located towards the centre of the beads.

Current investigations are completing these observations.

Optimising the culture of NC cells in vitro could further help us to expand these cells in larger scale for therapeutic purposes.

Keywords

Intervertebral disc degeneration; Notochordal cells

Abstract **#101**

MIMICKING NATURAL GRADIENTS IN 3DP SCAFFOLDS FOR OSTEOCHONDRAL REGENERATION

Ivo Beeren$_P$[1], Sandra Camarero-Espinosa[1,2], Piet Dijkstra[1], Carlos Mota Domingues[1], Ravi Sinha[1,3], Matthew Baker[1], Lorenzo Moroni[1]

[1] Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

[2] Polymer Science and Technology Department, Faculty of Chemisty, University of the Basque Country, Donostia-San Sebastian, Spain

[3] Division of Biomechanics, Department of Mechanical Engineering, KU Leuven, Leuven, Belgium

Corresponding author's email: i.beeren@maastrichtuniversity.nl

Once a lesion is present in the articular cartilage, the lesion is likely to deteriorate into whole joint osteoarthritis if not diagnosed timely. Tissue engineers have been designing scaffolds to regenerate osteochondral lesions at an early stage and minimize further degeneration of the joint. However, most constructs were designed under the assumption that the osteochondral tissue has a discontinuous stratified structure, whereas in reality the tissue gradually changes from cartilage into bone. Therefore, we hypothesized that for functional osteochondral regeneration a more biomimicking scaffold is needed, in which seeded cells smoothly differentiate from a chondrogenic towards an osteogenic phenotype across the construct. For this purpose, we developed 3D printed (3DP) scaffolds that contain a continuous gradient of chondro- or osteogenic inducing peptide on the fiber surface for gradual differentiation of human mesenchymal stromal cells (hMSCs). We used an in-house developed print head to extrude and mix two custom-made terminally functionalized polycaprolactone, onto which we specifically attached peptides or other biologically active molecules. Here, validation of the chemical system and fiber functionalization will be shown via different physico-chemical assays. Furthermore, we show with immunohistochemistry and biochemical assays that we exploit the gradient to manipulate hMSC behavior by attaching biological functionalities to the fibers such as (non-) cell adhesive or peptides. In a subcutaneous rat in-vivo study, we showed minimal toxicity towards our base materials and high cellular infiltration in the scaffolds. Finally, we implanted constructs in a rabbit osteochondral defect model and we aim to show histological results of this study.

Keywords

Material gradient; hMSCs

Abstract **#102**

STRATEGIES TO ENHANCE AND ACCELERATE THE PRODUCTION OF CELL-DERIVED MATRICES

Dimitrios Zeugolis$_P$[1,2]

[1] *REMODEL, NUI Galway, Galway, Ireland*

[2] *REMODEL, USI, Lugano, Switzerland*

Corresponding author's email: dimitrios.zevgolis@ucd.ie

AbstractCell-based tissue engineering therapies rely on the intrinsic capacity of cells to produce tissue-specific extracellular matrix (ECM) and create tissue-like surrogates in vitro. Although the efficiency and efficacy of cell-assembled tissue engineering therapies have been demonstrated clinically, only a handful of products have been commercialised. This limited technology transfer has been attributed to the prolonged ex vivo culture time required to develop a three-dimensional living substitute that is associated with phenotypic drift and very high manufacturing costs. Recent reports suggest that strategies that enhance and accelerate native ECM synthesis and deposition must be integrated into the developmental cycle of advanced therapy medicinal products to bridge the gap between positive therapeutic outcomes and market success. To this end, the concept of macromolecular crowding has been introduced that not only controls cell fate during ex vivo culture but also enhances (up to 120-fold) and accelerates (within 2-4 days) ECM deposition in permanently differentiated and stem cell cultures. This talk will provide an overview of the fundamentals of macromolecular crowding in eukaryotic cell culture for the accelerated development of functional and truly three-dimensional ECM-rich tissue like surrogates in vitro.References1. Satyam et al. Macromolecular crowding meets tissue engineering by self-assembly: A paradigm shift in regenerative medicine. Adv Mater. 2014;26(19):3024-3034AcknowledgementsThis work has received funding from the ERC under the EU's H2020 research and innovation programme grant agreement No. 866126; SFI, Career Development Award, grant agreement No. 15/CDA/3629; and SFI / European Regional Development Fund, grant agreement No. 13/RC/2073.

Keywords

extracellular matrix; cell derived matrices; advanced therapy medicinal products

Abstract **#104**

BIOPRINTING: ADVANCES, CHALLENGES, AND PERSPECTIVES ON CLINICAL TRANSLATION

James Yoo[P][1]

[1] *Wake Forest Institute for Regenerative Medicine*

Corresponding author's email: jyoo@wakehealth.edu

Over the years, various engineering strategies have been developed and applied for building functional tissues and organs for clinical applications. While techniques developed for tissue engineering and regenerative medicine applications have had initial successes in building a number of tissues clinically, challenges still exist in developing complex tissue systems. In recent years, 3D bioprinting has emerged as an innovative tool that enables rapid construction of complex 3D tissue structures with precision and reproducibility. This rapidly developing field promises to revolutionize the field of medicine addressing the dire need for tissues and organs suitable for surgical reconstruction. In this session novel and versatile approaches to building tissue structures using 3D bioprinting technology will be discussed. Clinical perspectives unique to 3D printed structures will also be discussed.

Abstract #105

CELL-FREE TISSUE REGENERATION

James Yoo[P][1]

[1] *Wake Forest Institute for Regenerative Medicine*

Corresponding author's email: jyoo@wakehealth.edu

Cell-based approaches have offered new opportunities for repairing various tissue pathologies. While the concept of using cells and scaffolds to develop functional tissues has remained as a viable strategy to bring new therapies to the clinic, this approach usually requires a donor tissue biopsy and extensive cell manipulation ex vivo prior to application in vivo. Simplifying these processes would provide a more efficient means of developing biological substitutes for functional tissue restoration. It has been demonstrated that almost every tissue in the body contains some type of stem or progenitor cells. These cells are believed to be part of underlying regenerative machinery that is responsible for daily maintenance and repair of injured tissue. The presence of an underlying regenerative mechanism in the form of tissue-specific stem and progenitor cells suggests that there may be a potential opportunity to bias the host response towards repair and replacement of tissue defects. This may be achieved by maneuvering host stem and progenitor cells using target-specific scaffolds. This emerging concept of in situ tissue regeneration using the body's own biological resources has gained increasing attention recently. In this session, translational tissue applications using this concept will be discussed.

Abstract **#106**

PRODUCTION, STERILIZATION AND IMPLANTATION OF HUMAN TISSUE-ENGINEERED BLOOD VESSELS PRODUCED BY WEAVING THREADS OF CELL-ASSEMBLED EXTRACELLULAR MATRIX.

Potart Diane$_P$[1]

[1] *INSERM*

Corresponding author's email: diane.potart@inserm.fr

To meet the clinical need for small vascular graft, we propose a new human and textile-based Tissue-Engineered Blood Vessel (TEBV). Vessels were produced by weaving yarn of Cell-Assembled extracellular Matrix (CAM) synthetized in vitro by skin fibroblasts. Study objectives were to 1) find the best sterilization method to simplify TEBVs production while preserving its biomechanical and biological properties, and 2) assess the human TEBV functionality in an immunodeficient rat model.
Well-organized human CAM sheets were produced in vitro after two months of culture with 20% bovine serum and 500 mM ascorbate. Sheets were cut into 5 mm wide ribbons that were sterilized using gamma irradiation (dry or wet, with high or low dose rates), electron beam irradiation, ethylene oxide (EtO), or supercritical carbon dioxide (scCO2). Ribbons (16 cm long, n=8/condition) were implanted subcutaneously in immunodeficient rats (4/rat) and tensile tests were performed after 2, 4, 12, and 24 weeks. TEBVs (8 mm long, 1.6 mm inner diameter) were woven from 2 mm-wide ribbons and implanted in abdominal aortas of immunodeficient rats.
Before implantation, the strength of the gamma-wet group was significantly lower than control (sterile production group) by 34%, while the EtO group was stronger (+20% of control). After five months, the gamma-wet group remained the weakest, while scCO2 group seemed to be stronger than other conditions. Preliminary surgeries in rat abdominal aortas demonstrated graft implantability and the absence of transmural leakage.
In conclusion, ribbons can be sterilized while preserving satisfying mechanical properties, and be assembled into promising grafts.

Abstract **#107**

A NOVEL STRATEGY FOR LONGER TERM DIFFERENTIATION OF HUMAN EMBRYONIC TISSUES FROM PLURIPOTENT STEM CELLS AND SIMULATIONS OF TERATOMA FORMATION IN VITRO

Alejandro Hidalgo Aguilar[P][1], Lucy Anne Smith[1], Stefan Alexander Przyborski[1,2]

[1] *Durham University*

[2] *Reprocell Europe*

Corresponding author's email: Alejandro.Hidalgo-Aguilar@durham.ac.uk

Human pluripotent stem cells (hPSCs) have become an alternative model for recapitulating key milestones of human embryogenesis such as tissue development, which is an important and highly regulated process, yet poorly understood1. Currently, creating an in vitro model that provides the appropriate microenvironmental cues in a spatiotemporal manner to enable hPSCs to differentiate into complex 3D tissue structures remains a challenge2,3. Our initial studies have shown that mature tissue-like structures derived from three germ layers can be generated in vitro using a combination of murine embryoid bodies (mEBs) followed by their prolonged maintenance and differentiation on a porous scaffold. This approach extends EB viability by flattening the shape of the EB resulting in reduced diffusion distances and increased cell viability that subsequently permits long-termer cultures, increased cell differentiation and the formation of high order structures. Current work involving size controlled human embryoid bodies (hEBs) has revealed the ability of the system to maintain and form complex tissue structures with features of various human embryonic tissues. We hypothesize that through the development and application of new in vitro technologies, it is feasible to extend the growth and maturation of differentiating tissues derived from hPSCs. Furthermore, the manipulation of physical and chemical factors within the microenvironment will enable the ability to direct tissue development in specific ways. In conclusion, we anticipate that this novel approach will provide an opportunity to study early stages of tissue development and deliver an animal free alternative to assess the pluripotency of hPSCs.

Keywords

Human pluripotent stem cells; 3D culture; Embryoid body

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Abstract **#109**

REGENERATIVE REHABILITATION: FROM PROTEINS TO PATIENTS

Martin Stoddart$_P$[1]

[1] *AO Research Institute Davos*

Corresponding author's email: martin.stoddart@aofoundation.org

Regenerative Rehabilitation is an emerging area that aims to enhance regenerative medicine. One definition is "Regenerative rehabilitation integrates regenerative technologies with rehabilitation clinical practices to restitute function and quality of life in individuals with disabilities due to otherwise irreparable tissues or organs damaged by disease or trauma".[1]It is well established that musculoskeletal system healing is highly regulated by the mechanical environment experienced after injury. Thus offering an ideal opportunity to enhance tissue regeneration. The AO Foundation is a nonprofit organization dedicated to improving the care of people with musculoskeletal injuries through research, development, education and quality assurance in the principles, practice, and result of fracture treatment. Since 1958 early mobilization has been a central tenant of AO principles of patient treatment. More recently, the underlying cellular mechanisms are being increasingly understood, allowing for optimization of rehabilitation protocols. Within this presentation applied methods will be discussed at the protein and at the tissue level, where mechanical load is demonstrably the main driver of bone healing. Localized cellular responses can be driven at the protein level by changes in expression due to the mechanical forces applied. Additionally, it has been shown that physical stimulation is able to activate latent TGF-β by way of mechanical load alone. Once the underlying process is understood, the translation from defined preclinical environment to a less defined clinical environment remains a challenge. Therefore, how this information can be used to inform development of clinical devices will be discussed, with an example provided.

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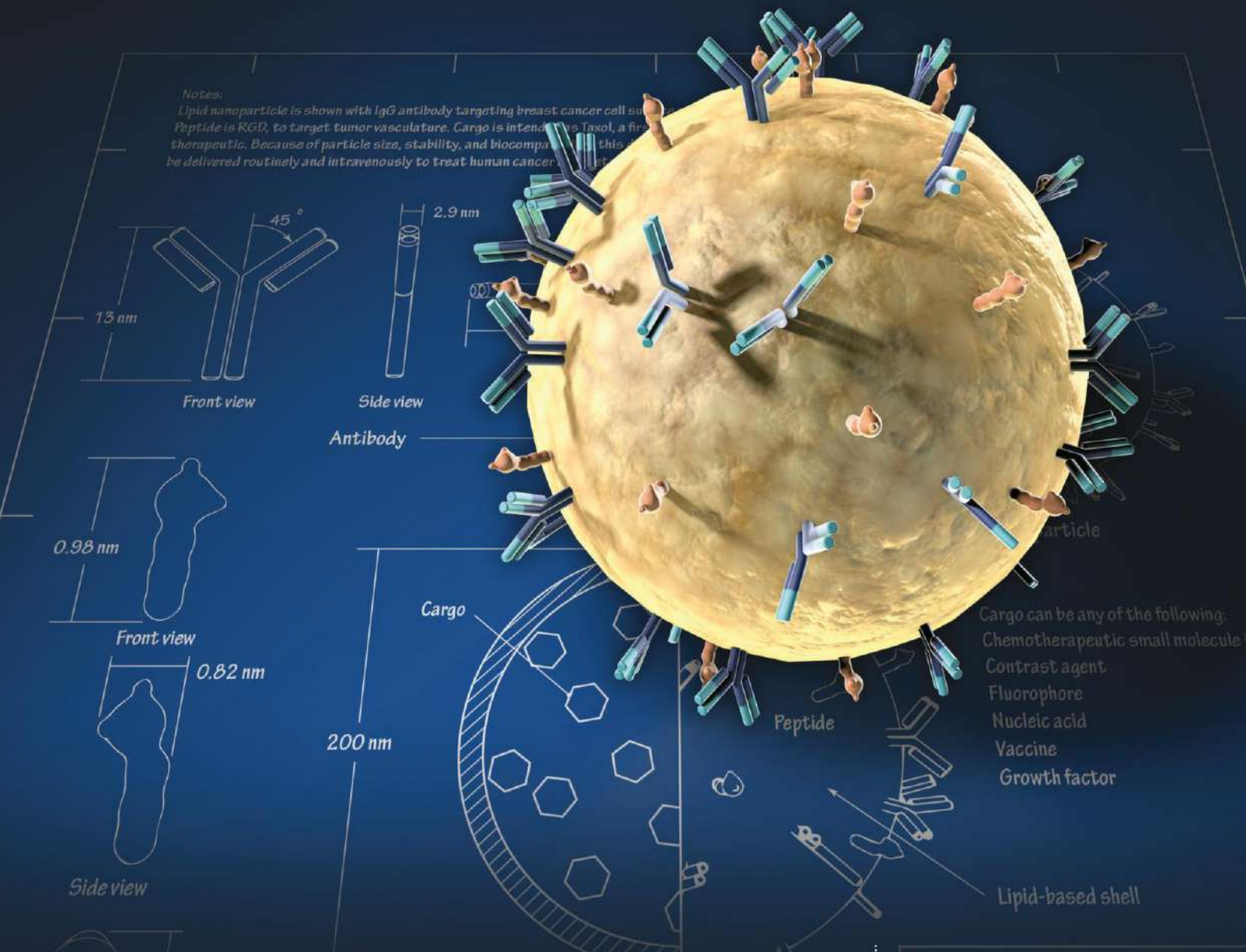

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Abstract **#111**

MULTIPLE TRAITS OF HUMAN NASAL SEPTUM-DERIVED CHONDROCYTES ENCAPSULATED IN COLLAGEN AS INJECTABLE THERAPEUTIC AGENT FOR CARTILAGE DEFECT REPAIR

SeHwan Hwang$_P$[1]

[1] *The catholic university of Korea*

Corresponding author's email: yellobird@catholic.ac.kr

Implantation of chondrocytes is a promising therapeutic strategy for the treatment of cartilage repair. However, articular cartilage injury has poor repair ability and has limited regeneration capacity by therapy based on implantation of articular chondrocytes. Human chondrocytes from nasal septal cartilage (hNCs) are an excellent alternative source of articular chondrocytes for cartilage tissue regeneration because hNCs can be obtained easily by minimally invasive collection procedures and chondrogenic properties of hNCs are maintained after extensive culture expansion, which are demonstrated by immunostaining and expression of cartilage-specific protein. In this study, we fabricated hNCs by encapsulating them in hydrogel, type I collagen, to transplant into injured articular cartilage tissue, and we evaluated their characteristics. Cell moving after encapsulation was detected by microscopy and hNCs were uniformly distributed throughout the collagen. hNCs encapsulated in collagen (hNCs-collagen) showed higher viability than 80% and remain at this levels for 12 hr at 4°C incubation. Fluorescence staining of hNCs-collagen showed great expression levels of chondrocyte-specific protein, Type II collagen, Sox9 and Aggrecan in clinically applicable condition of 24 hr. Notably, in osteochondral defect model, implantation of hNCs-collagen led to greater chondrogenic repair into focal cartilage defect in rats. Moreover, at 8 weeks after implantation, many of human cells were detected around cartilage defect, which is expected to potential and benefits for cartilage regeneration. hNCs-collagen would be a valuable therapeutic agent for implantation into injured cartilage tissue and can be used clinically for repair of cartilage defect in the knee.

Abstract **#112**

EFFICACY OF PRECONDITIONED OR GENETICALLY MODIFIED IL4-SECRETING MESENCHYMAL STROMAL CELLS IN A MODEL OF STEROID-ASSOCIATED OSTEONECROSIS OF THE FEMORAL HEAD IN RABBITS

Stuart Goodman[P][1,2], Masahiro Maruyama[3,4], Seyedsina Moeinzadeh[3], Roberto Guzman[3], Michiaki Takagi[4], Yunzhi Peter Yang[5]

[1] *Department of Orthopaedic Surgery and Bioengineering,*

[2] *Stanford University*

[3] *Department of Orthopaedic Surgery, Stanford University*

[4] *Department of Orthopaedic Surgery, Yamagata University Faculty of Medicine, Yamagata, Japan*

[5] *Departments of Orthopaedic Surgery, Material Science and Engineering, Bioengineering, Stanford University School of Medicine, Stanford, CA*

Corresponding author's email: masa.maruyama1460@gmail.com

INTRODUCTION: Bone healing can be augmented by preconditioning MSCs (pMSCs) with inflammatory cytokines. Another approach is timely resolution of inflammation using immunomodulatory cytokines. We investigated the efficacy of pMSC and genetically modified MSCs that over-express IL-4 (IL4-MSCs) on early stage steroid-associated osteonecrosis of the femoral head (ONFH) in rabbits.METHODS: 36 male mature NZW rabbits received methylprednisolone acetate (20mg/kgIM) 4 weeks before surgery. There were 6 groups: 1. Core Decompress (CD) alone – a 3 mm drill hole+ injection of:2. hydrogel (HG) - 200 µl of hydrogel carrier3. MSCs–1 million rabbit MSCs4. pMSC - LPS (20 µg/ml) + TNFα (20 ng/ml) preconditioned MSCs 5. IL4-MSCs – rabbit IL-4 over-expressing MSCs 6. IL4-pMSCs – preconditioned IL-4 over-expressing MSCsEight weeks after surgery, femurs were evaluated by microCT, biomechanical, and histological analyses.RESULTS: Bone mineral density (BMD) and bone volume fraction (BVF) increased outside the CD in the pMSC group compared to the CD and MSC groups ($p < 0.05$). IL4-pMSC group was increased compared to the CD group ($p < 0.05$). The percentage of empty lacunae in the IL4-MSC group was significantly less than other groups outside the CD ($p < 0.05$); however, IL4-MSC group had less trabecular bone formation inside the CD. DISCUSSION: pMSC increased new bone formation after CD in ONFH; IL4-MSCs decreased the number of empty lacunae. Immunomodulation of bone healing has the potential to improve bone healing after CD for early stage ONFH; these interventions must be applied in a temporally sensitive fashion.

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Abstract **#113**

APPLICATION OF SECRETOME FROM HYPOXIA-PRECONDITIONED MESENCHYMAL STEM CELLS ON CARTILAGE REGENERATION

Yanmeng Yang[P][1], Yingnan Wu[1]

[1] *Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore*

Corresponding author's email: e0338264@u.nus.edu

Mesenchymal stem cell (MSC) secretome-based therapy is regarded as a promising treatment for cartilage lesions due to the action of MSC secretory factors. Hypoxia, as the physiological condition of MSCs, increases its proliferation, survival and paracrine activity, and has been shown to enhance angiogenesis, migration and suppress inflammation in various disease models. In this study, we investigate the effect of conditioned medium (CM) generated from hypoxia-preconditioned human bone marrow MSCs on cartilage regeneration. MSCs were subjected to normoxic (20% O2) or hypoxic (1% O2) conditions to generate CM. Treatment with hypoxic CM enhanced the proliferation and migration of both MSCs and chondrocytes. Hypoxic CM exerted a superior effect over normoxic CM in the relief of IL-1β induced up-regulation of pro-inflammatory genes (COX2, IL-6) and hypertrophic marker, COLX in chondrocytes exposed to IL-1β. In addition, extracellular matrix degrading enzyme, ADAMTS5 was significantly suppressed by hypoxic CM, resulting in the rescue of IL-1β induced degradation of extracellular matrix. Furthermore, protein array analysis revealed increased production of chemokine (SDF1), anti-inflammatory factors (IL1-ra and GDF15) and growth factors (VEGF, HGF and FGF2) in hypoxic CM relative to normoxic CM, correlating to the functional augmentation of hypoxic CM. Taken together, the results demonstrate that hypoxic condition was able to significantly potentiate the migration and proliferation capability, as well as the chondro-protective and anti-inflammatory paracrine function of MSC secretome. Hypoxia could be a more efficient culture platform to improve the repertoire of MSC secretome for cartilage regeneration.

Abstract **#114**

BIOMECHANICS OF BONE TISSUE REGENERATION IN 3D POROUS STRUCTURES: A MECHANO-DRIVEN APPROACH

Maria Angeles Perez$_P$[1], Gabriele Nasello[1], Simone Russo[1], Jose Manuel García-Aznar[1]

[1] *University of Zaragoza*

Corresponding author's email: angeles@unizar.es

Large bone defects represent a clinical challenge for which regenerative therapies and tissue engineering strategies aim at offering treatment alternative to conventional replacement approaches by metallic implants (1). Materials currently used for bone tissue scaffold fabrication are inorganic materials (magnesium, titanium, ceramics) and natural or synthetic polymers (write examples of both polymers, e.g. PCL). Some of them possess an interesting characteristic, that is degradability. Additionally, 3D printing technologies provide porous scaffolds with designed shape, controlled chemistry and interconnected porosity, where bone will regenerate (2).

In the last decades a strong effort has been made to optimize scaffold designs by means of computational tools. because the final aim is to promote bone regeneration with mechanobiologically optimized scaffolds. In fact, computer techniques and mathematically based models are becoming very useful tools for material engineers and biologists to advance the understanding of the scaffold behaviour under different environments (3). Regeneration algorithms elucidated the relationship between the tissue being formed within the pores and the loading environment, as well as the mechanical benefits of a degrading scaffold during bone formation (4).

In this work, a mechano-driven model is presented which recapitulates the mechanics of 3D printed porous scaffolds (non-degradable and degradable) and predicts short- and long-term bone regeneration. The scaffold regenerative potential based on the biomechanical contributions of both the host and the scaffold itself is also evaluated. Using this computational model, we demonstrated that the mechanical stimulus is intrinsically associated with the regenerative response to bone scaffolds in terms of bone formation.

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Abstract **#115**

THE BEST OF BOTH WORLDS: IN VITRO EVALUATION OF COMBINING TWO STEM CELL TYPES FOR TRUE CARDIAC REPAIR

Hanne Beliënp[1], Lize Evens[1], Marc Hendrikx[2], Virginie Bito[1], Annelies Bronckaers[1]

[1] *Biomedical Research Institute (BIOMED), Hasselt University, Diepenbeek, Belgium*

[2] *Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium*

Corresponding author's email: hanne.belien@uhasselt.be

Myocardial infarction (MI) irreversibly destroys millions of cardiomyocytes in the ventricle, making it the leading cause of heart failure worldwide. Current therapies are unable to replace the lost cardiac tissue, thereby not preventing progression towards heart failure. As a novel regenerative therapy, a new cardiac stem cell type with high cardiomyogenic differentiation capacity, the cardiac atrial appendage stem cell (CASC), was identified. Although CASCs can improve cardiac function after MI, the application potential of CASCs is limited by the low cell engraftment and survival upon transplantation, largely due to their limited angiogenic properties. Since dental pulp stem cells (DPSCs) are highly angiogenic but lack cardiomyogenic properties, they can be used as a priming strategy to improve the efficacy of regenerative CASC therapy. This study investigated whether DPSCs are able to enhance CASCs properties in vitro by examining the effect on CASCs proliferation, viability, migration and angiogenesis. Rat CASCs were either cultured in conditioned medium of rat DPSCs or indirectly co-cultured with rat DPSCs. The effect of DPSCs on CASCs proliferation and viability was assessed by a propidium iodide assay in presence of 2% serum or serum-free medium respectively. Migration of CASCs was examined using a transwell migration assay. To investigate the effect on CASCs paracrine angiogenic properties, conditioned medium of treated and untreated CASCs was collected for in vitro functional assays using a human umbilical vein endothelial cell line (HUVEC). Endothelial cell proliferation, migration and tube formation were assessed. In ovo angiogenesis was examined using the chorioallantoic membrane assay.

Keywords

Preconditioning; Cardiac stem cells; Dental pulp stem cells

Abstract **#117**

TARGETING EARLY HEALING PHASE WITH TITANIA NANOTUBE ARRAYS ON TUNABLE DIAMETERS TO ACCELERATE BONE REGENERATION AND OSSEOINTEGRATION

Yin Xiao[P][1]

[1] *Queensland University of Technology*

Corresponding author's email: yin.xiao@qut.edu.au

Blood coagulation and inflammation are the earliest biological responses to implant surfaces. It is well known that implant nano-surfaces can significantly impact the osteogenesis and osseointegration, which is most likely through the influence on the early phase of bone regeneration. However, the interplay between blood clot property and inflammatory reaction on nanosurfaces is less understood. This study investigated the influence of distinct surface properties of titanium on blood clot features, and whether the adaptable clot features are capable of steering osteoimmunomodulation targeting osteointegration. Titania nanotube arrays (TNAs) with different diameter were fabricated and in vitro evaluation with the whole blood indicated that TNA with a diameter of 15 nm (TNA-15) enabled noteworthy platelet activation resulting in distinct clot features compared with that of pure Ti and TNA with a diameter of 120 nm (TNA-120). The co-culture of macrophages (MΦs) with the clot showed that the clot on TNA-15 downregulated the inflammation and manipulated a favorable osteoimmunomodulatory environment for osteogenesis. In the animal model, TNA-15 downregulated inflammation-related markers (IL-1β, TNF, and IL8), while upregulated growth metabolism-related gene expression (BMP-2, WNT5A, and ITG-β1) in early healing hematoma identified by RNA sequencing. Eight weeks post-implantation in rabbit model TNA-15 promoted the de novo bone formation with improved extending of osteocyte dendrites, demonstrating the desired osseointegration. The results indicate that surface nano-dimensions can significantly influence blood clot formation, which manipulates a favorable osteoimmunomodulatory environment for bone regeneration and osseointegration.

Abstract **#116**

GUIDED TISSUE ASSEMBLY AND BIOFABRICATION OF MACRO-SCALED EPITHELIAL ORGANOID TUBES

Yan Yan Shery Huang[P][1], Ye Liu[1], Catherine Dabrowska[1], Antranik Mavousian[1], Bernhard Strauss[1], Fanlong Meng[2], Eugene Terentjev[1], Joo-Hyeon Lee[1]

[1] *University of Cambridge*

2 Chinese Academy of Science

Corresponding author's email: yysh2@cam.ac.uk

Epithelial organoids cultured in appropriate 3D conditions typically develop a micro-scaled, cystic structure lined with a polarized epithelium. Despite their great potential in research and therapy, epithelial organoids grow in heterogeneous sizes, and are too small to display physiologically relevant performance and applications. Here, we show guided assembly of mouse tracheal basal stem cell organoids towards geometrically-defined, lumenized constructs. The observed shape stability of organoid assembly is confirmed by theoretical modelling based on organoid morphology and the physical forces involved in fusion. We provide hypothesis on how epithelial organoid assembly can be achieved in a more efficient and predictable manner, of which principles could be extended to other organoid types developed form epithelial stem cells. The guided self-assembly strategy presented here opens up the possibility for biofabricating size-relevant, geometrically defined epithelial structures towards broad applications in biomimetic organoid-on-a-chip, and tissue engineering.

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Liu Y, et al. doi.org/10.1002/advs.202003332

Abstract **#118**

MODELLING OF CELL INVASION OF SCAFFOLD AT THE INITIAL STAGE OF SEEDING

Ziyu Liu[P][1,2], Shanshan Yuan[3], Liqi Ng[2], Chaozong Liu[2]

[1] *Beihang University*

[2] *University College London*

[3] *Qingdao Municipal Hospital*

Corresponding author's email: ziyu.liu.16@ucl.ac.uk

Cell seeding process influence the final tissue formation [1,2]. Preceding all other steps of tissue engineering, better cell adhesion and even spatial distribution are associated with improved culture results [3]. For understanding cell seeding process deeply, two geometry scaffold were designed to investigate the cell attachment process through experiment and simulation analysis, including a cubic design and a truncated octahedron design . A novel numerical model is developed by coupling the volume of fluid (VOF), discrete phase model (DPM) and cell impingement model (CIM) for predicting cell distribution after cell seeding. This methodology could help to predict initial stage of cell attachment clinical test more accuracy and also reduce a number of in vivo experiments.This method is able to predict the cell distribution and assessing the scaffold design. Truncated octahedron scaffold showed a more even distribution than cubic design in vitro cell seeding. Truncated octahedron scaffold with spatial distribution beams could provide a more suitble environment for nurtients transport and cell movement and distribution.

Keywords

Cell seeding; Computational Fluid Dynamics; Osteochondral scaffold

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Abstract **#119**

SILK BASED ELECTROACTIVE BIOMATERIALS FOR NEURAL APPLICATIONS

Rajiv Borah$_P$[1], Joseph Christakiran[2], Biman Behari Mandal[2]

[1] *Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam, India*

[2] *Indian Institute of Technology, Guwahati*

Corresponding author's email: rajivb6007@gmail.com

Accelerating the rate of nerve regeneration can result in a better functional outcome for the denervated tissue. One of the potential methods for improving nerve regeneration and the restoration of function is electrical stimulation (ES). This inspires to exploit electrically conductive neural scaffold to promote neurite outgrowth. We have developed biocompatible and biodegradable 3D aligned electroconductive scaffolds with minimal immune response for possible use as smart nerve guidance channels (NGCs). The proposed smart NGCs were fabricated using natural fibroin protein of B. mori and A. assama silk (endemic to North-East India). To confer speedy axonal growth, we introduced electrically conducting polymer, polypyrrole (PPy), in the scaffold. The hypothesis of speedy axonal growth in primary dorsal root ganglions (DRGs) and enhanced neural differentiation of adipose derived mesenchymal stem cells (ADMSCs) was tested under pulsed ES using a function generator at amplitude 0, 50, 100, 200, and 300 mV/cm (500 Hz) for 2h/day (3 days). Current-voltage and CV analysis demonstrated their voltage dependent conductive behaviour and redox stability in neutral electrolyte, including cell culture media. The ES test showed multiple fold increase in axonal growth in DRGs and enhanced neural differentiation of ADMSCs. The materials showed minimal immune response when evaluated with murine macrophage cells for release of IL1-beta and nitric oxide. With the higher redox stability cell culture media and faster axonal growth as well as improved neural differentiation, our findings could be considered as a proof-of-concept for a new NGC for faster nerve repairing.

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Abstract **#120**

LOW-TEMPERATURE VACUUM-EVAPORATION AS A DEVITALISATION AND PRESERVATION TECHNIQUE FOR LONG-TERM STORAGE AND AMBIENT DISTRIBUTION OF TRANSPLANTABLE HUMAN CORNEAS

Laura E Sidney[1], Emily R Britchford[1,2], Owen D McIntosh[1], Andrew Hopkinson[1,2], Lydia J. Beeken[3]

[1] *Academic Opthalmology, Division of Clinical Neuroscience, University of Nottingham, UK*

[2] *NuVision Biotherapies Ltd, Nottingham, UK*

[3] *Academic Ophthalmology, Division of Clinical Neuroscience, University of Nottingham, Nottingham, UK*

Corresponding author's email: lydia.beeken@nottingham.ac.uk

Global demand for corneal tissue was 12.7 million patients in 2016 with approximately 185,000 corneal transplants performed annually in 116 countries, leaving over 12 million corneal-blind cases worldwide untreated [1]. One limitation is access to quality donor tissue due to inadequate eye donation services and infrastructure in some less-economically developed countries. This is compounded by the fact that there are no long-term storage solutions for effectively preserving spare donor corneas collected in countries with a surplus. We have developed a low temperature vacuum evaporation preservation technique that can dehydrate human corneas to enable ambient storage of corneas for over 2 years and allows for global ambient shipping. Dried corneas are comparable to non-dried in all aspects (weight, thickness, ECM content and structure), other than a loss of cellular viability during drying, rendering the preserved corneas as devitalised. When implanted subcutaneously in rats, the dried corneas were well tolerated, with cellular migration into the matrix and no visible immune rejection. We spoke to 12 corneal consultants, at 7 different hospitals, all gave positive feedback regarding future use and suitable clinical indications of tectonic support and deep anterior lamellar keratoplasty were identified. Our preservation technique provides a non-viable, dehydrated, cornea alternative, suitable for a range of clinical indications and tectonic support in emergency situations. It can be stored on the shelf in hospitals for over 2 years and can be shipped at ambient temperatures worldwide, relieving the global shortage of corneal tissue.

Keywords

Cornea; Transplant

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Abstract **#121**

CLICK SMALL INTESTINAL SUBMUCOSA HYDROGELS FOR VOCAL FOLD TISSUE ENGINEERING

Mika Brown$_P$[1], Shirley Zhu[2], Maryam Tabrizian[1], Nicole Li-Jessen[3,1]

[1] *Department of Biomedical Engineering, McGill University, Montréal, Canada*

[2] *Department of Microbiology and Immunology, McGill University, Montréal, Canada*

[3] *School of Communication Sciences and Disorders, McGill University, Montréal, Canada*

Corresponding author's email: mika.brown@mail.mcgill.ca

Severe vocal fold (VF) defects significantly impact patients' communication and swallowing functions.[1] Existing injectable biomaterials can temporarily augment the defected VF. However, they are absorbed into surrounding tissue over time without repairing the defect, leading to a need for re-injection. Hydrogels produced from decellularized extracellular matrices (dECM) such as porcine small intestinal submucosa (SIS) have been shown to provide cues that aid in wound healing but degrade rapidly and possess low mechanical integrity.[2, 3] To address these challenges, we used click chemistry to develop a SIS-alginate composite hydrogel that possesses greater stability than SIS alone, without a deleterious impact on cells.[4] We first used carbodiimide crosslinking to functionalize the SIS with norbornene and alginate with methyltetrazine and achieved a degree of substitution of approximately 5% in both cases. Gelatin was used as a control for SIS. When mixed, the functionalized SIS and alginate underwent a click reaction, tetrazine ligation, and formed a gel within one hour. Through gene ontology of SIS proteomics, we found that the hydrogel contains proteins involved in cellular adhesion as well as the reconstruction of the extracellular matrix (ECM), key processes in VF regeneration. Specifically, these proteins promote pathways in fibroblast-mediated ECM remodeling, breaking down damaged or scarred ECM and replacing it with healthy neo-ECM. Our results indicate that the click SIS-alginate hydrogel provides cues that could aid in stimulating complete tissue regeneration, thereby reducing the medical burden of biomaterial rejection in the treatment of VF defects.

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Abstract **#122**

IN VITRO MODEL OF THE IMMUNOMODULATORY EFFECT OF WHARTON'S JELLY DERIVED MESENCHYMAL STEM CELLS (WJ-MSCS) IN PULMONARY FIBROBLASTS SPHEROIDS INFECTED WITH HUMAN CORONAVIRUS 229E (HCV 229E).

Fabian Rho Mas[P][1], Fabio Gonzalez Sanchez[2], Rebeca Garcia-Varela[3], Beni Camacho-Perez[2], Ana Luisa Camacho-Torres[2], Jose Navarro-Partida[1], Juan Pablo Aguilar Alemán[3,2]

[1] *Tecnologico de Monterrey, School of Medicine and Health Science*

[2] *Top Health S.A.P.I de C.V.*

[3] *Tecnologico de Monterrey, School of Engineering and Science*

Corresponding author's email: jp.aguilar@tec.mx

Patients infected with the SARS-Cov2 virus have been severely affected [1]. The high rate damaged reported by the effects of this virus is due mainly to a storm pro-inflammatory cytokines production that is triggered by immune response overexpression [2]. Thus, research on this virus has become a high priority for public health [3]. However, biosafety regulations hinder rapid advancements on this research line. Therefore, it is essential to find an in vitro model that provides a close approximation to the actual SARS-Cov2 infection.

The developed model is based on the detection of anti-inflammatory cytokines produced by Wharton's jelly derived mesenchymal stem cells (WJ-MSCs) as a response of HCV-229E infection at a lung spheroid. For this purpose, a co-culture of lung fibroblast spheroid (MRC-5) constituted in human collagen type 1, infected with HCV-229E, and human peripheral blood leukocytes were grown for 3 days, pursuing the generation of the pro-inflammatory signaling cascade. Subsequently, WJ-MSCs were co-cultured within the previous described environment.

As a result, it was demonstrated that WJ-MSCs play an immunomodulatory role against HCV-229E infection. After 72 h of WJ-MSCs exposure a significant decrease in pro-inflammatory cytokines IL(1,2,6,7) and TNF α was observed. Additionally, a very marked increase in anti-inflammatory cytokines such as IL(4,10,11,13) and TGF-β was detected. We conclude that this model could be used for COVID-19 research and other similar respiratory syndromes without the manipulation of the actual virulent strand, this would aid in the understanding of mechanism behind the disease and the use WJ-MSCS such as therapy.

Keywords

In vitro model; Spheroids; Stem Cells

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Abstract **#123**

TYPE 2 IMMUNE RESPONSE AND SKIN TISSUE ENGINEERING

Behrouz Farhadihosseinabadi[P][1], Mozhdeh Mohammadian[2], Ameneh Jafari[3], Meghdad Abdollahpour-Alitappeh[4], Mazaher Gholipuormalekabadi[5], Koushan Sineh Sepehr[6], Ali Samadikuchaksaraei[5], Hassan Niknejad[7]

[1] *Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2] *Department of Hematology and cell therapy, Tarbiat Modares University, Tehran, Iran*

[3] *Proteomics Research Center, School of Allied Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[4] *Cellular and Molecular Biology Research Center, Larestan University of Medical Sciences, Larestan, Iran*

[5] *Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran*

[6] *Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran*

[7] *Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences*

Corresponding author's email: beh_far1364@yahoo.com

Treating of full-thickness wounds has long been considered by researchers. Full-thickness wounds require medical attention to prevent infections and scarring. In such group of skin injuries, trying to increase the epithelialization rate and wound closure is paramount importance. Tissue engineering has provided various strategies to repair such skin damages. One of the possible strategies is recruiting immune system to accelerate the healing process. Type 2 immune response consists of different cells and growth factors that help wounds to be healed faster by increasing parameters that support wound healing, such as increasing angiogenesis and wound contraction. Cytokines including IL-10, IL-4, IL-5, and TGF-B are the main cytokines in this type of immune. The interaction of these factors together causes the macrophages to polarize towards type 2 macrophages, and these cells increase epithelialization and wound closure by secreting different repairing factors such as VEGF, ARG-1, and TGF-B. So far, various substances have been identified with the ability to induce type 2 immune response. Oligosaccharides derived from parasitic antigens and human milk have the property of inducing a type 2 immune response. The use of these materials in skin substitutes in addition to reduce the inflammation of the damaged area, accelerates healing by shifting immune response toward the type 2 immunity.

Keywords

Type 2 immune responses; Skin ; Tissue engineering

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Abstract **#124**

SUCCESSFUL RAT-TAIL REPLANTATION USING REDV-CONJUGATED DECELLULARIZED MICROVASCULAR GRAFTS AS BOTH ARTERIAL AND VENOUS SUBSTITUTES

Hiroki Yamanaka[P][1,2], Naoki Morimoto[2], Atsushi Mahara[1], Tetsuji Yamaoka[1]

[1] *Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute*

[2] *Department of Plastic and Reconstructive Surgery, Graduate School of Medicine and Faculty of Medicine, Kyoto University*

Corresponding author's email: ymnkahrk@kuhp.kyoto-u.ac.jp

Background: Acellular-matrix based microvascular prostheses have potential as an alternative to autologous-venous grafts utilized in 2–30% of free flaps and replantation surgeries, but methods are required to improve their blood interfaces. Here, the applicability of a decellularized microvascular graft modified with the integrin α4β1 ligand, REDV, for arterial and venous reconstruction was evaluated in a rat-tail replantation model.

Methods: Male-ACI-rat tail arteries (inner diameter: 0.6 mm) were decellularized via ultra-high-hydrostatic pressure treatment, and modified with REDV to induce antithrombogenic interfaces and promote endothelialization post-implantation [1]. Grafts were implanted into the tail artery and vein to re-establish blood circulation in amputated Lewis-rat tails (n =12). The primary endpoint was the survival rate of replants, analyzed by laser-speckle imaging. Secondary endpoints were graft patency, remodeling, and regeneration for 6 months.

Results: Three cases of technical errors or postoperative self-mutilation were excluded. All remaining nine tails survived without partial necrosis. Six-month Kaplan-Meier patency was 100% for arterial grafts and 62% for venous grafts. Two venous grafts were occluded for 3 weeks but displayed no sign of tail-venous congestion, suggesting that the implants remained patent until the regeneration of collateral veins. At 6 months, the neo-tunica media was regenerated inside the neo-intima. One arterial graft extracted at 6 months displayed drug-induced smooth muscle contraction in vitro.

Conclusions: Rat-tails were successfully replanted using allogenic REDV-conjugated microvascular grafts as both arterial and venous substitutes. Further investigations using larger animal models are required.

Keywords

decellularized; microvascular graft

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Abstract **#125**

IMPROVING SPINAL FUSION WITH INJECTABLE HYDROGELS

David Owen[P1], Simon Partridge[1], Joseph Snuggs[1], Anthony Michael[2], Ashley Cole[1], Neil Chiverton[1], Mark Wilkinson[3], Chris Sammon[1], Christine Le Maitre[1]

[1] *Sheffield Hallam University*

[2] *Sheffield Teaching Hospitals*

[3] *University of Sheffield*

Corresponding author's email: david.owen@sheffield.ac.uk

INTRODUCTION: Current clinical treatment for spinal instability requires invasive IVD fusion with a lumbar cage and pedicle screw instrumentation. We previously reported a novel injectable hydrogel (Bgel), which supports the delivery and differentiation of mesenchymal stem cells (MSCs) to bone forming cells(1) and supports bone formation in vivo(2). Here, we tested the application of this gel which contains hydroxyapatite nanoparticles and investigated its bone forming capacity. METHODS: Laponite crosslinked pNIPAM-co-DMAc incorporated with hydroxyapatite nanoparticles (Bgel), was injected into lumbar cages and bovine and human IVD tissue explants, with and without MSCs. Human IVD samples from discectomy patients were obtained from Sheffield Teaching Hospitals. All samples were cultured for 4 weeks. Tissue samples were cultured under hypoxia (5%) in standard culture media. Micro-CT scans were analyzed to determine mineralization within the lumbar cages. All samples were embedded to wax for histological and immunohistochemical analysis. RESULTS: Bgel seeded with MSCs showed mineralization within the lumbar cages. Increased collagen deposition was seen in tissue explants injected with Bgel, with evidence of elevated native cell migration towards the hydrogel. Increased collagen staining was seen in explants injected with MSC-incorporated Bgel. Calcium deposition was increased in Bgel-treated samples, compared to controls. Phenotypic markers; runx2, collagen type X, osteopontin and osteocalcin were assessed to determine the bone synthesizing response within the explant cultures. DISCUSSION: We have developed an injectable hydrogel system which shows potential to support spinal fusion either via directing bone growth through the center of bone cages, or via direct injection into the disc.

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Abstract **#126**

RECYCLING CULTURE MEDIUM FOR ENHANCED CELLULAR INTERACTIONS IN IN VITRO BONE MODELS

Michelle A.M. Vis[P][1], Keita Ito[1], Sandra Hofmann[1]

[1] Orthopaedic Biomechanics, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, PO Box 513, 5600 MB, Eindhoven, the Netherlands

Corresponding author's email: m.a.m.vis@tue.nl

In a standard cell culture, medium is exchanged. By doing so, important soluble factors for auto- and paracrine signaling are lost and the cells must make a new effort to restore their communication through soluble factors [1]. This effort could considerably influence the cell's behavior. However, renewal of the medium is necessary to prevent waste product accumulation, which in turn inhibits cell growth [2].

To overcome the waste accumulation problem, cell culture systems were developed in which culture medium was filtered or dialyzed to remove waste products. At the moment, dialysis for the re-use of culture medium is not frequently reported and mainly used for culturing of human induced pluripotent stem cells [3-5], which require the use of expensive macro-molecules. Recycling medium enabled the reduction of these costly macro-molecules [5].

This study investigates whether dialysis could be used to improve an osteogenic differentiation culture. A custom-made simple dialysis culture system with a cellulose dialysis membrane is used. It is hypothesized that recycling of culture medium contributes to a more efficient and more physiological environment for cell proliferation and differentiation, leading to faster osteogenic differentiation as auto- and paracrine factors are not removed from the culture. Results show significantly higher ALP/DNA in the dialysis group compared to the control after 3 weeks, which indicates a faster differentiation in the dialysis group. This is confirmed by a higher osteopontin expression after 4 weeks. Overall, the dialysis system shows promising potential as a tool for faster and more physiological osteogenic differentiation.

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Abstract **#127**

LOSARTAN SUPPRESSES TNF-ALPHA INDUCED INFLAMMATORY RESPONSE AND DEGENERATION OF HUMAN NUCLEUS PULPOSUS CELLS

Babak Saravi[1,2], Judith Pfannkuche[1,2], Sonja Häckel[3], Mauro Alini[1], Sibylle Grad[1], Norbert Südkamp[2], Hagen Schmal[2], David Kubosch[2], Zhen Li[1], Gernot Lang[2]

[1] *AO Research Institute Davos, Davos, Switzerland*

[2] *Department of Orthopedics and Trauma Surgery, University Hospital Freiburg, Freiburg, Germany*

[3] *Inselspital, Department of Orthopedic Surgery, University of Bern, Switzerland*

Corresponding author's email: babak.saravi@jupiter.uni-freiburg.de

INTRODUCTION: Low back pain (LBP) is the main reason for disability worldwide, leading to an immense socioeconomic burden for the affected individuals and healthcare systems. Degeneration of the intervertebral disc (IVD) is known to be a major cause of LBP. We recently showed that effector molecules of the tissue renin-angiotensin system (tRAS) were expressed in the human IVD and that their expression correlated with the extent of inflammatory responses and tissue degeneration[1]. We, therefore, sought to investigate the impact of the angiotensin II type 1 receptor blocker (AGTR1) Losartan on TNF-alpha induced inflammation and degeneration of the human nucleus pulposus (NP) cells.METHODS: Human NP tissue was obtained from male trauma patients (n=4). NP cells were isolated and expanded. TNF-alpha (10ng/ml) was used to induce degeneration and inflammation. Losartan (1mM) was added, and gene expression of tRAS molecules and inflammatory markers were measured. T0070907, a PPARγ antagonist, was applied to the NP cells to investigate the regulatory pathway of Losartan.RESULTS: Losartan significantly suppressed TNF-alpha induced expression of inflammatory mediators (Nitric oxide, TNF-alpha), tRAS related molecules (Angiotensinogen, Angiotensin-Converting-Enzyme, Cathepsin D), and catabolic enzymes (MMP-1, MMP-3). These inhibitions of TNF-alpha induced proinflammatory and degenerative processes were counteracted by T0070907, indicating Losartan attenuates TNF-alpha induced inflammation and degeneration acting in part as a PPARγ agonist.CONCLUSIONS: Collectively, these data illustrate that Losartan, an AGTR1 inhibitor, suppresses TNF-alpha induced inflammation and degradation in human NP cells. Furthermore, our findings reveal that Losartan unfolds this effect, at least in part, through the PPARγ pathway.

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Abstract **#128**

DEVELOPMENT OF ELECTROCONDUCTIVE BIOMATERIAL SCAFFOLD FOR SPINAL CORD INJURY REPAIR

Aleksandra Serafin$_P$[1], Mario Culebras Rubio[1], J. Miguel Oliveira[2,3], Maurice N. Collins[1]

[1] *Bernal Institute, School of Engineering, University of Limerick, Ireland*

[2] *3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Guimarães, Portugal*

[3] *ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal*

Corresponding author's email: Aleksandra.Serafin@ul.ie

Spinal cord injury (SCI) is a debilitating disorder that often results in the loss of motor and sensory function of the patient, as well as paralysis at or below the injury site. Currently, there is no effective treatment for SCI, with most of the clinical endeavors focusing on rehabilitation of the patient [1]. In the past, biomedical engineering strategies for SCI repair focused on the utilization of neural stem cells and biomolecules, though a new tissue engineering strategy is currently emerging in the form of electroconductive materials, which often deliver more optimal results when equated to their non-conductive counterparts. In developing such a material, critical requirements must be met; including excellent biocompatibility, biodegradability, and suitability for cell/bioactive factor incorporation, whilst allowing multiple pathways for conducive facilitation of neuronal or axonal tract regeneration in the spine. In the present work, biomaterials with all the above features are developed and characterized for suitability as an electroconductive biomaterial. We established a novel synthesis method to produce Poly(3,4-ethylenedioxythiophene) (PEDOT) electroconductive nanoparticles based on the mini-emulsion method, which possess the potential to produce nanoparticles with a myriad of functionalities. Further incorporation of PEDOT nanoparticles with bioactive components allowed for the development of a novel electroconductive biomaterial. The physicochemical characterization of this biomaterial includes morphology, degradation profiles, compression tests, electroconductivity, rheology, and biocompatibility studies. In-vitro cytocompatibility studies include Alamar Blue assay and Immuno-fluorescence staining of protein markers on neural stem cells.

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Abstract **#129**

SCALING AND OPTIMISING OPERATING CONDITIONS FOR THE CULTURE OF MYOBLASTS IN HOLLOW FIBRE BIOREACTORS

Scott J Allan$_P$[1,2], Paul A De Bank[3], Marianne J Ellis[2]

[1] *Centre for Sustainable Chemical Technologies, University of Bath, BA2 7AY, UK*

[2] *Department of Chemical Engineering, University of Bath, Bath, BA2 7AY, UK*

[3] *Department of Pharmacy & Pharmacology, University of Bath, Bath, BA2 7AY, UK*

Corresponding author's email: sja39@bath.ac.uk

The nascent field of cultured meat, as a proposed low value, high throughput product, requires the implementation of large-scale tissue engineering to take advantage of economies of scale and meet potential product demands. Limitations to scale exist for the adherent culture of skeletal muscle cells in bioreactors on microcarriers as a result of the inherent shear forces imposed.[1–2] An alternative, high cell density perfusion bioreactor option that decouples the cells from the shear force of liquid flow was investigated in the form of hollow fibre bioreactors, by assessing the methods for scaling up and optimising the flow conditions for nutrient supply.

Porous polystyrene hollow fibres were fabricated in-house by dry-wet spinning and used as scaffolds in custom hollow fibre bioreactors to expand C2C12 cells with lumen media flow (DMEM + 10% FBS). Monitoring was performed via discrete dissolved oxygen measurement with in-line sensors and off-line media sample analysis. Cell number and viability was quantified and used to analyse expansion, cell density, growth kinetics and metabolite kinetics.

Variation of inlet flow conditions enabled the effect of flow regimes and mass transfer on cell growth, metabolite utilization and final cell density to be assessed. Different methods of scaling hollow fibre bioreactors were investigated. i) Scaling by increasing bioreactor size, performed via in situ cell dissociation and seeding in larger bioreactors. ii) Numbering up (scale-out), verified by the operation of multiple bioreactors in parallel. iii) Process intensification, by decreasing the size of the hollow fibres to increase the maximum cell density attainable.

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Abstract **#130**

CHARACTERIZATION OF CHITOSAN/POLYVINYL ALCOHOL-BASED HYDROGEL WOUND DRESSING FILMS LOADED WITH EXOSOMES EXTRACTED FROM HUMAN ADIPOSE-DERIVED STEM CELLS, SILVER NANOPARTICLES, AND SILDENAFIL CITRATE

Ali Samadi Lp[1,2], Saed Azandeh[2], Mahmoud Orazizadeh[2], Vahid Bayati[2], Mohammad Rafienia[3], Masoud Ali Karami[4], Akram Gavahi[2]

[1] *Department of Anatomy and Embryology, School of Medicine, Bam University of Medical Sciences, Bam, Iran*

[2] *Cellular and Molecular Research Center (CMRC), Department of Anatomical Science, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran*

[3] *Biosensor Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

[4] *Nanotechnology Research center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Corresponding author's email: alisamadi23@yahoo.com

Trauma and burn wounds have a bad prognostic nature. Hydrogels are the best candidates for dressing such wounds. Exosomes are extracellular vesicles responsible for generating intercellular signaling pathways and cell growth stimulation. Sildenafil citrate has been reported to be effective in angiogenesis. Accordingly, the purpose of this study was to make a novel hydrogel wound dressing following the integration of various findings on wound healing and the use of regenerative medicine.

Materials and methods: Various compounds were fabricated by chitosan/polyvinyl alcohol and then characterized to obtain the optimal composition using several techniques, including a water vapor passage test, antibacterial test, TEM, SEM, water absorption, tensile strength, biodegradability, and FTIR. Then, the synthesized silver nanoparticles (AgNPs) were added to the compound. Exosomes from hADSCs were characterized. Sildenafil citrate reached 5% by weight suspension. Dressings with different compounds were applied to four Wistar rat groups. Hematoxylin and eosin (H&E), silver, and Mallory's trichrome staining methods were used to examine the tissue samples.

Results: The findings revealed the optimal dressing ratio. Better antibacterial activity was found for the AgNPs dressing. The tissue-extracted cells were confirmed to be stem cells using real-time PCR for oct4 and Nanog genes. The presence of exosomal markers was verified by a Western blot test. A novel approach for preparing an exosome for TEM imaging has been developed, and acceptable recovery was observed for the rats. A significant improvement was found for the dressing group within two weeks.

Conclusion: Our new fabricated dressing exhibited satisfactory scarless wound healing properties.

Abstract **#131**

MACROPHAGE REPROGRAMMING FOR WOUND HEALING APPLICATIONS

Moein Amoupour$_P$[1,2], Ali samadikuchaksaraei[3,2], Peiman Brouki Milan[3,2], Mahmood Barati[1,4], Davoud Ahmadvand[5]

[1] *Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran*

[2] *Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran*

[3] *Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran*

[4] *Shahid Beheshti University of Medical Sciences, School of Pharmacy, Department of Pharmaceutical Biotechnology, Iran*

[5] *School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran*

Corresponding author's email: moein.amoupour.ap@gmail.com

Macrophages play key roles in wound healing process(1). In response to different stimuli, macrophages can adopt the polarization into pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes. Non-healing chronic wounds, such as diabetic ulcers, indefinitely arrest in the inflammation stage of wound healing. Due to the presence of inflammatory cytokines, local macrophages remain in the M1 phenotype and the normal phases of healing do not make any progress(2). While various therapeutic strategies are used to accelerate wound healing, recent studies target the microenvironmental signals and molecular mechanisms that mediate macrophage polarization(3).

In this study, using the native macrophage intracellular signaling pathways and regulators, we investigated the effect of SOCS protein family on macrophage polarization. We designed antisense oligodeoxynucleotide (ASO) sequences against SOCS3 to induce sequence-specific gene silencing by targeting SOCS3 mRNA(4). Mannosylated chitosan (MCH) was prepared as a carrier to delivery SOCS3 ASO(5). The cellular uptake, transfection efficiency, and repolarization capability of RAW 264.7 macrophages were assessed. Transfection of MCH/SOCS3 ASO decreased SOCS3 gene expression and also increased M2 phenotype gene expression in RAW264.7 macrophages, which could reprogram the macrophages from M1 to M2 phenotype.

We investigated the effect of ASO on macrophages phenotype in the impaired wound healing in diabetic mice. Based on histological and immunohistochemical studies it accelerated wound closure, decreased collagen deposition and increased the M2 phenotype levels, compared to the untreated group. Overall,our results demonstrated that MCH/ASO led macrophage functional polarity from M1 toward an anti-inflammatory M2 phenotype, which could be a promising platform for chronic wound healing.

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Abstract **#132**

POLYHYDROXYALKANOATES, NATURAL MATERIALS OF BACTERIAL ORIGIN, IDEAL FOR CARDIAC TISSUE ENGINEERING

Ipsita Roy[P][1,2], Prachi Dubey[3], Qasim Majid[4], Annabelle Fricker[1], David Gregory[1], Christopher Grigsby[5], Molly M Stevens[6,5], Cesare Terracciano[2], Sian E Harding[2]

[1] *Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK*

2 National Heart and Lung Institute, Faculty of Medicine, Imperial College London, UK

3 University of Westminster, London, UK

4 NHLI, Imperial College London, London, UK

5 Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

6 Department of Bioengineering, Department of Materials, IBME, Faculty of Engineering, Imperial College London, UK

Corresponding author's email: royips@gmail.com

Polyhydroxyalkanoates (PHAs), a family of bioresorbable and biocompatible polymers, are known to be particularly cardio-regenerative in nature(1,2,3). Myocardial infarction results in the generation of scar tissue with limited regeneration due to the modest nature of intrinsic myocardial regenerative capability. The concept of a cardiac patch is tailored to meet the unmet medical need of cardiac regeneration where a biomaterial-based patch would be used to induce efficient cardiac regeneration. Medium chain length PHAs (MCL-PHAs) are highly elastomeric in nature and have been shown to be excellent substrates for the growth and function of neonatal cardiomyocytes(3). This work describes an in-depth study of the potential of MCL-PHAs for the development of functional cardiac patches laden with human pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) and human embryonic stem cell derived endothelial cells (hESC-ECs), providing mechanical support, vascularisation and cell-based therapy.The hiPSC-CMs attach and grow well on MCL-PHA scaffolds, with growth and beat rates comparable to that on gelatin. Immunohistochemical analysis has shown that the hiPSC-CMs exhibit all expected phenotypic characteristics of mature, adult cardiomyocytes when grown on the PHA scaffolds, with the required sarcomere length. Calcium transients, the main functional assay of the hiPSC-CMs are within required values, confirming that the MCL-PHAs support calcium exchange and promote contractions. Similar results were observed on melt electrowritten 3D substrates using hiPSC-CMs and hESC-ECs. Recently, 3D-printed MCL-PHA based multi-material structures have been created and found to be equally functionally efficient. Overall, this work established the highly cardio-regenerative nature of MCL-PHAs, natural materials of bacterial origin.

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Abstract **#133**

POLYSACCHARIDE-POLYPLEX ULTRATHIN COATINGS FOR NANONEEDLE-BASED GENE DELIVERY

Daniel Hachim$_P$[1], Juzhi Zhao[2], Jash Bhankharia[2], Hyejeong Seong[1], Liliana Brito[2], Christopher Grigsby[3], Michele Becce[1], Molly Stevens[1,2,3]

[1] *Department of Materials, Institute of Biomedical Engineering. Imperial College London, London, UK.*

[2] *Department of Bioengineering, Institute of Biomedical Engineering. Imperial College London, London, UK.*

[3] *Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.*

Corresponding author's email: d.hachim-diaz@imperial.ac.uk

Nanoneedles are a successful and attractive alternative for gene delivery, without eliciting cell and tissue toxicity [1, 2]. Although nanoneedles offer exceptional localized tissue specificity, control over dosing and release kinetics is poor, as mediated only by surface adsorption. To overcome these limitations, ultrathin coatings were developed using multilayer film deposition of chitosan and polysaccharide-based polyanions onto the surface of silicon nanoneedles. A pCAG-GFP gene plasmid was loaded into pABOL polyplexes and incorporated within the coating multilayers, containing one or more polyplex layers. Confocal microscopy, SEM and XPS surface analyses revealed that all coating components, including polyplexes were uniformly distributed on the surface of nanoneedles, preserving the architecture of nanoneedles. Degradation of the coating and polyplexes over time was monitored with AFM. Release assays showed that an increased number of layers containing polyplexes provided an increased amount and length of pCAG-GFP release. In vitro testing on COS-7 cells seeded on coated nanoneedles showed improved cell viability (close to 100%) compared to non-coated nanoneedles. Transfection efficiency on COS-7 cells was studied via fluorescence microscopy and flow cytometry using increasing layers of polyplexes and multiple nanoneedle patterns. Results 24-hour post-transfection revealed increased transfection efficiency when increasing number of layers. Interestingly, distinct transfection efficiencies where observed with different nanoneedle patterns. These results suggest that transfection efficiency is mostly dependent on the surface area available for interaction with cells, rather than nanoneedle-mediated puncture of cell membrane. Regardless of the mechanisms mediating transfection, these coated nanoneedle-based platforms represent a promising alternative for controlled gene delivery.

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Abstract **#135**

A 3D PRINTED MICROFLUIDIC BIOCHIP FOR RAPID DRUG SCREENING

Yi-Chen Li$_P$[1]

[1] *Department of Chemical Engineering, Feng Chia University*

Corresponding author's email: ycli0212@gmail.com

Microfluidic devices offer a relatively rapid, compact and low-cost alternative for mimicking the physiological environment and can test various drug samples with extremely small reagent volumes. Therefore, the development of high-throughput microfluidic chip for culturing cells that can accurately and efficiently predict overall efficacy of drugs. However, traditional lithography methods including stencil-assisted patterning, microfluidic patterning and others exist limitations such as complex manufacturing procedures, limited patternable flexibility, vulnerability of patterning equipment, etc. that are difficult to provide a process to fabricate a high-throughput chip on the substrates with various surface properties which are suitable for culturing cells. Here we propose a concept to fabricate a 3D tree-shape like microfluidic biochip on the substrates with various surface properties (i.e., hydrophilic and hydrophobic) by using a 3D printing technology. We hypothesized that this 3D bioprinted microfluidic chip could provide a rapid easy process to fabricate a microfluidic chip on different substrates and offers a concentration gradient for high-throughput screening drug effects on cells.

Keywords

3D printing; microfluidic biochip; drug screening

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Abstract **#137**

REDUCED GRAPHENE OXIDE: POTENTIAL FOR CARDIAC TISSUE ENGINEERING

Nafiseh Baheiraei[P][1]

[1] *Tissue Engineering and Applied Cell Sciences Division, Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

Corresponding author's email: n.baheiraei@modares.ac.ir

Graphene based nanomaterials ,with unique electrical and mechanical properties, are encouraging candidates to support the electrical conductivity of cell-seeded scaffolds for cardiac tissue engineering.

This research was designed to investigate the physicochemical and biological effects of an electroactive cardiac patch of collagen (Col) being coated covalently by different concentrations of graphene oxide (GO). Some samples were also reduced to rGO by a reduction agent to restore the high conductivity of GO. The expression profiles of the cardiac markers were analyzed using quantitative real-time PCR. Vascularization potential of the prepared scaffolds was investigated through subcutaneous implantation of the scaffolds in mice. Also, the antibacterial properties of reduced scaffolds against Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes were confirmed by culture and Field Emission Scanning Electron Microscope (FESEM) observation.

By increasing GO concentration, both mechanical properties and Electrical conductivity of the scaffolds were increased. No toxic effects were observed against human umbilical vein endothelial cells (HUVECs) using MTT assay. Compared with the Col counterpart, electroactive containing rGO scaffolds upregulated cardiac genes, including Cx43, Actin4, and Trpt-2.The obtained results also confirmed the angiogenic and antibacterial properties of reduced Go-containing scaffolds for cardiovascular applications.

Taken together, our results showed that rGO coating provides promising properties to Col scaffolds presenting a desirable micro environment for cardiomyocytes coupling and gene upregulation as well as angiogenic and antibacterial activities for cardiac patch application.

Keywords

Graphene Oxide; Cardiac patch; Electroactive

Abstract **#139**

MODEL-FREE ASSESSMENT OF BIOMECHANICAL PROPERTIES FOR PERSONALIZED MEDICAL DEVICES

Michael Gasik[P][1]

[1] *Aalto University Foundation*

Corresponding author's email: michael.gasik@aalto.fi

Biomaterials along with engineered tissues and implants are essential part of any operations for tissue repair and regeneration. Mechanical signal transduction is one of the fundamental modes of cells and materials surfaces interaction but it has so far received insufficient attention. This is also critical for personalized implants (such as 3D fabricated with a complex geometry) and for the regulatory approval. Existing different methods of biomechanical testing give rather different outcomes, and it is not easy to obtain realistic, true properties, evaluated in a correct and physiologically relevant way.

For the risk assessment and regulatory compliance with MDR 2017/745 it is important to deploy methods which provide model-free, invariant and robust estimation of the factors and properties equally useful for medical engineers as well as clinical applications. Here we present an overview of new methodology with examples, compliant to MDR Annex I, enabling robust design and tailoring implants for musculo-skeletal applications.

A part of this work has received funding from the EU Horizon 2020 research and innovation programme under grant No 760921 "PANBIORA".

Abstract **#140**

BIOMECHANICAL CHARACTERIZATION OF VERTEBRAL BODY REPLACEMENT IN SITU: EFFECTS OF DIFFERENT FIXATION STRATEGIES

Enchun Dong[P][1], Jianfeng Kang[2], Dichen Li[1], Ling Wang[1]

[1] *Xi'an Jiaotong University*

[2] *Jihua Laboratory*

Corresponding author's email: dongenchun@stu.xjtu.edu.cn

Artificial vertebral implant with a lateral or posterior screw-rod fixation system are usually employed to rebuild the lumbar spine after spinal resection. This study aims to evaluate the influence of different surgical fixation strategies on the biomechanical performance of a reconstructed lumbar spine system. Two typical lumbar spine reconstruction case that correspond to lateral or posterior fixation systems were selected. Finite element analyses were performed, and comparisons were made between the two models in terms of the safety and stability under various normal daily activities. The load from the upper vertebral body can be effectively transmitted onto the lower vertebral body with the lateral fixation system; which was favorable for bone growth. However, significantly high stresses were concentrated around the interaction region between the screws and bone, owing to the uneven lateral fixation structure; this may increase the risk of bone fractures and screw loosening. For the posterior fixation case, stably posterior fixation structure was favorable to maintain stability for the reconstructed lumbar spine. However, owing to the stress shielding effect, the load was mainly transmitted via the posterior fixation rod rather than the vertebral implant. Therefore, the predicted strain on the endplate were insufficient for bone ingrowth under most of the spinal activates, which could cause bone loss and prosthesis loosening. The results of this study will have a clear impact in understanding the biomechanics of the lumbar spine with different fixation strategies and providing necessary instructions to the design and application of the lumbar spinal fixation system.

Abstract **#141**

CADAVERIC CELLS AS BUILDING BLOCKS FOR TISSUE ENGINEERING: SPINAL CORD NEURONAL PROGENITORS.

Andrey Mikhailov$_P$[1], Yoshiyuki Sankai[1]

[1] *University of Tsukuba*

Corresponding author's email: mikhailov.andrey.fp@u.tsukuba.ac.jp

Until recently, neuronal tissue was not considered for donations from adults, other than for research purposes. Most of the published works utilized one of two main sources of neuronal stem cells (NSCs) for interventions: established cell lines and iPSC-derived NSCs. Cadaver derived live cells should be able to reproduce all the “bystander” neurodegenerative effects, and, in principle, give advantages of better immune matching, highest available cell number, and lower costs associated with in-laboratory cell propagation at required scale.

We studied distribution of apoptotic cells in goat spinal cord at different (0-56hr) intervals postmortem. Number of TUNEL-positive cells found in histological samples and amount of activated Caspase 3 fragment measured by Western Blot gradually increased over time, however, were much lower that in brain of the same animals. Distribution of GD2-positive cells (neuronal progenitors, NSCs) was the densest in the anterior horns of the spinal cord grey matter. NSCs were separated by magnetic force from homogenised and enzymatically processed spinal cord with antibodies against GD2, while the mature neurones with antibodies against CD24. Separated cells were placed on multielectrode arrays and their electrical activity measured before and after enforced differentiation. Cells differentiated from NSCs displayed close spontaneous activity pattern as the matured neurones and displayed similar response to GABA stimulation with bicuculline. We conclude that NSCs can be successfully harvested from post mortal spinal cord material and can be differentiated into electrically active functional neurones.

Keywords

cadaveric; neuronal progenitors; affinity separation

Abstract **#144**

NIDOGEN-1 MITIGATES ISCHEMIA AND PROMOTES TISSUE SURVIVAL

Aline Zbinden[P1], Shannon Layland[1], Max Urbanczyk[1], Daniel A. Carvajal Berrio[1,2], Julia Marzi[1,3], Monika Zauner[1], Markus Templin[3], Arjun Deb[4,5,6], Garry P. Duffy[7], Gay M. Crooks[5,8,9], Johannes A. Eble[10], Hanna K. A. Mikkola[4,5,11], Ali Nsair[5,12], Martina Seifert[13,14], Katja Schenke-Layland[1,3,12]

[1] Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany

[2] Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University Tübingen, Germany

[3] Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany

[4] Department of Molecular, Cell and Developmental Biology, UCLA, Los Angeles, CA, USA

[5] Eli & Edythe Broad Stem Cell Research Center, UCLA, Los Angeles, CA, USA

[6] Molecular Biology Institute, UCLA

[7] Anatomy & Regenerative Medicine Institute, School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Ireland

[8] Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, CA, USA

[9] Department of Pathology & Laboratory Medicine, and Pediatrics, David Geffen School of Medicine at UCLA, UCLA, Los Angeles, CA, USA

[10] Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Münster, Germany

[11] Molecular Biology Institute, UCLA, CA, USA

[12] Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

[13] Institute of Medical Immunology, Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health (BIH), Berlin, Germany

[14] Charité Universitätsmedizin, Berlin Institute of Health (BIH) Center for Regenerative Therapies Berlin (BCRT), Germany

Corresponding author's email: aline.zbinden@uni-tuebingen.de

Ischemia impacts multiple organ systems and is the major cause of morbidity and mortality in the developed world. Ischemia disrupts tissue homeostasis, driving cell death and damaging tissue structure integrity. Strategies to heal organs, like the infarcted heart, or to replace cells, as done in pancreatic islet β-cell transplantations, are often hindered by ischemic conditions. Here, we discover that the basement membrane glycoprotein nidogen-1 attenuates the apoptotic effect of hypoxia in cardiomyocytes and pancreatic β-cells via the αvβ3 integrin and promotes a regenerative immune response. We show that nidogen-1 significantly increases heart function and angiogenesis, while reducing fibrosis, in a mouse post-myocardial infarction model. These results demonstrate the protective and regenerative potential of nidogen-1 in ischemic conditions.

Keywords

Ischemia; Nidogen-1

Abstract **#145**

MOULDED OR 3D PRINTED MEDICAL SILICONES: BIOMECHANOLOGICAL EVALUATION

Alexandra Zühlke[P][1], Michael Gasik[1], Nihal Engin Vrana[2], Celine Blandine Muller[3], Julien Barthes[3], Yevgen Bilotsky[4], Edwin Courtial[5], Christophe Marquette[6]

[1] *Aalto University Foundation*

[2] *Spartha Medical SAS*

[3] *INSERM*

[4] *Seqvera Ltd.*

[5] *3dFAB*

[6] *3dFAB*

Corresponding author's email: michael.gasik@aalto.fi

Modern 3D printing of implantable devices provides an important opportunity for the development of personalized implants with good anatomical fit. 3D printing of silicone has been challenging and recently is possible via extrusion. Potential impacts of the 3D printing process of silicone on its biomechanical and biological properties has not been yet studied so in this study we compare 3D printed and molded silicone for their cytotoxicity, surface roughness, biomechanical properties and in vivo tissue reaction. The 3D printing process found to increase nanoscale roughness and microscale topography. Neither the presence of these features nor the differences in processes were found to result in an increase in cytotoxicity or tissue reaction for 3D printed structures, exhibiting limited inflammatory reaction and cell viability above the threshold values. On the contrary, the biomechanical properties have demonstrated significant differences in static and dynamic conditions, and also in thermal expansion. Our results demonstrate that 3D printing can be used for establishing a better biomechanical microenvironment for the surrounding tissue of the implant particularly for fragile soft tissue like epithelial mucosa without having any negative effect on the cytotoxicity or in vivo reaction to silicone. For engineering of the implants, however, one must take into account the differences in mechanical properties to result in correct and personalized geometry and proper physical interaction with tissues.

This work has received funding from the EU Horizon 2020 research and innovation programme under grant No 760921 "PANBIORA".

Abstract **#146**

AGGREGATES OF EQUINE MESENCHYMAL STEM CELLS WITH GELATIN HYDROGEL FOR REGENERATIVE THERAPY OF TENDON INJURY

Norihisa Tamura[P][1], Hiroshi Mita[1], Kentaro Fukuda[1], Taisuke Kuroda[1], Fumio Sato[1], Atsutoshi Kuwano[1], Yasuhiko Tabata[2], Yoshinori Kasashima[1]

[1] *Japan Racing Association Equine Research Institute*

[2] *Institute for Frontier Life and Medical Sciences, Kyoto University*

Corresponding author's email: tamura@equinst.go.jp

We hypothesized that the implantation of mesenchymal stem cells (MSCs) aggregates with gelatin hydrogel microspheres (GMS) enhances the efficacy of cell therapy for equine superficial digital flexor tendon (SDFT) injury. We evaluated the residual period of the administered MSC and their therapeutic effect in vivo.

1. Labelled MSC were used to produce aggregates with GMS or monolayer-cultured MSCs. Each of them was injected into a contralateral injured SDFT for six horses. The fluorescent intensity was measured after 10 days. 2. After the injections performed in the same manner for another six horses, ultrasonography examinations were performed to evaluate healing of the lesion over time.

As a results, 1. The fluorescence intensity obtained from aggregates-administered tendons was significantly higher than that from tendons treated with the monolayer-cultured cells ($p<0.05$). 2. Compared to tendons treated with the monolayer-cultured cells, the aggregates-administered tendons had an early decrease in the ratio of hypoechogenic area to the cross-sectional tendon area, an early increase in the strain ratio of sonoelastography (each $p<0.01$) and an earlier disappearance of new blood vessels ($p<0.05$).

Our results suggest the aggregates with GMS are effective to prolong the residual period of MSCs in injured tendons. The findings of the ultrasonography examinations suggest an early recovery of tissue stiffness and early progression of the inflammatory process in the lesion. Although further research is necessary to establish a mechanism to improve the repair of injured tendon, aggregates with GMS would be effective to enhance the efficacy of cell therapy.

Keywords

horse; tendon; MSC aggregates

Abstract **#147**

CRISPR-MEDIATED EPIGENETIC MODIFICATION RESCUES OSTEOPOROTIC ASC CHONDROGENIC DEFICIENCY AND PROMOTES CALVARIAL BONE HEALING IN OSTEOPOROTIC RAT

Anh Vu Truongp[1], Ya-Hui Lin[1], Thi Kieu Nuong Nguyen[1], Mu-Nung Hsu[1], Yu-Chen Hu[1,2]

[1] *Department of Chemical Engineering, National Tsing Hua University, Hsinchu, Taiwan*

[2] *Frontier Research Center on Fundamental and Applied Sciences of Matters, National Tsing Hua University, Hsinchu, Taiwan*

Corresponding author's email: av.chemist@gmail.com

Calvarial bone regeneration poses a tremendous challenge in clinical settings due to poor spontaneous healing. Previous studies reported that chondrogenic induction of ASC was able to boost the healing progress via the non-native endochondral ossification pathway. Non-osteoporotic and osteoporotic ASC (OVX-ASC) are inferior in osteogenesis capacity and predisposed to adipogenic commitment, while OVX-ASC chondrogenic tendency remains elusive. CRISPR-mediated epigenetic manipulation has emerged as a robust approach for targeted gene activation/silencing. To activate chondrogenesis, we constructed a baculovirus encoding dSpCas9 fused with the histone acetylation domain p300core with (Bac-p300-VPR) or without (Bac-p300) an extra recruitable MCP-VPR activation fusion targeting the chondrogenic Sox trio (Sox5, Sox6, Sox9). To suppress adipogenesis, we generated a baculovirus encoding dSaCas9 fused with the DNA methyltransferase 3A (Bac-DNMT3A) targeting adipogenic regulators (C/ebpa, Pparg). Bac-p300-VPR conveyed more efficient gene activation than Bac-p300 while Bac-DNMT3A exerted considerable gene knockdown. Bac-p300-VPR-transduced ASC displayed a profound promotion of chondrogenic phenotype as judged by abundant presence of glycosaminoglycan (GAG), while OVX-ASC remained resistant to chondrogenic differentiation albeit the Sox trio activation. Strikingly, co-transduction of OVX-ASC with Bac-p300-VPR/Bac-DNMT3A substantially drove the cells towards chondrogenic lineage. 3D construct culture of the co-transduced OVX-ASC gave rise to significant amount of GAG and collagen type II over the mock-transduced construct. Ultimately, implantation of the transduced construct accelerated and enhanced calvarial defect regeneration in osteoporotic rats. Collectively, our results demonstrated the feasibility of CRISPR-based epigenetic alteration for orthogonal regulation of gene expression in stem cells for regenerative medicine.

Keywords

CRISPR; osteoporosis; calvarial bone healing

Abstract **#148**

INFLUENCE OF SERUM ALKALINE PHOSPHATASE ACTIVITY ON OSTEOGENIC DIFFERENTIATION

Sana Ansari[P][1,2], Keita Ito[1,2], Sandra Hofmann[1,2]

[1] *Orthopaedic Biomechanics, Department of Biomedical Engineering, Eindhoven University of Technology*

[2] *Institute for Complex Molecular Systems, Eindhoven University of Technology*

Corresponding author's email: s.ansari@tue.nl

Fetal bovine serum (FBS) is a common supplement in cell culture providing cells with nutrients but is controversial due to its unknown batch-dependent composition that could lead to significant differences in experimental outcomes1. For example, FBS has been shown to influence the formation of mineral precipitation and osteogenic differentiation of cells. It was hypothesized that alkaline phosphatase (ALP) could be a serum component which would affect the mineralization process2. Hence, this study focused on how the ALP activity of different FBS types influences in vitro bone-like tissue formation. Acellular and human bone marrow mesenchymal stromal cell-seeded silk fibroin scaffolds were cultured in medium containing four different FBS types under static and dynamic conditions (stirring-induced wall shear stress). Acellular constructs under static condition showed higher calcium phosphate content in the medium with higher serum ALP activity. Mineral deposition on acellular constructs were greatly influenced by dynamic conditions compared to static ones. The cell-seeded constructs were also influenced by the FBS type. In the statically cultured cell-seeded constructs, medium ALP activity had more impact on mineral precipitation than cell ALP activity; while, in the dynamic cultures cell ALP activity had larger contribution on mineral deposition. This study highlights that the ALP activity inherent to different FBS brands could be one of the driving components having an impact on the mineralization process and should be determined in batch testing. For future studies, conventional FBS should be replaced by defined or serum-free medium to increase the reproducibility of studies and comparability of obtained outcomes.

Keywords

Alkaline phosphatase ; Fetal bovine serum; Mineralization

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Abstract **#149**

BI-COMPONENT THERMO- AND PHOTO-SENSITIVE BIOINKS WITH TUNABLE PHYSICO-MECHANICAL PROPERTIES TO 3D BIOPRINT TISSUE ANALOGUES FOR REGENERATIVE MEDICINE PURPOSES

Gianluca Ciardelli[P][1], Arianna Grivet-Brancot[1], Stefano Calzone[1,2], Choudhury Deepak[3], May Win Naing[3,4], Monica Boffito[1]

[1] *Politecnico di Torino*

[2] *Dipromed srl (current address)*

[3] *Biomanufacturing Technology, Bioprocessing Technology Institute (BTI), 20 Biopolis Way, Singapore 138668*

[4] *Singapore Institute of Manufacturing Technology, 2 Fusionopolis Way, Singapore 138634, Singapore*

Corresponding author's email: gianluca.ciardelli@polito.it

The current innovation on rapid prototyping has increased the demand for customized bioinks with tunable properties for different biomedical applications. In this work, thermo-sensitive and photo-curable bioinks were developed, using an amphiphilic Poloxamer 407-based polyurethane (PEU) entrapped into a photo-crosslinked poly(ethylene glycol) diacrylate (PEGDA) mesh. PEU concentration within the bioink was optimized (12%w/v) to observe gelation around 37°C within few minutes. PEGDA was produced at two molecular weights (3350 and 6000Da) and blended with PEU at different ratios. Circular-shaped samples (10mm diameter, 2mm thickness) were then photo-crosslinked using UV light (365nm, 10mW/cm2, photo-initiator: lithium phenyl-2,4,6-trimethylbenzoylphosphinate, 0.05%w/v). In simulated physiological environment, PEGDA3350-containing systems dissolved after 1-2 weeks, while PEGDA6000-based gels were still present after two months. Rheological and photo-rheological characterization evidenced the possibility to finely tune gel mechanical properties working on PEU/PEGDA ratio and PEGDA molecular weight (storage modulus within the range 5-100kPa upon photo-curing). Bone marrow-derived mesenchymal stem cells were embedded into the formulations and 3D bioprinted (up to 8 layers) at high resolution (approx. 30 µm) exploiting bioink thermo-sensitivity to provide primary stability and then photo-crosslinked layer-by-layer to further stabilize the structures. Each step of the printing process (i.e., cell embedding, printing, photo-curing) was biologically optimized to minimize cell suffering. Viability tests suggested that the whole scaffold fabrication process did not induce cell death and cells exhibited prolonged viability and homogeneous distribution. The here designed thermosensitive and photocurable hydrogels thus represent a promising platform of bioinks with tunable physico-mechanical properties for driving stem cell differentiation towards the desired phenotype.

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Abstract **#151**

NOVEL ALLOGENIC COLLAGEN GRAFT FOR MINIMAL INVASIVE PROCEDURE FOR TYMPANIC MEMBRANE REGENERATION

Grecia Andrea Cardoso Hernández$_P$[1], Brenda Karen Aguillon-Estrada[1], Eduardo Miramontes-Beas[1], Beni Camacho-Pérez[1], Luis Renee González Lucano[2], Juan Pablo Aguilar Alemán[3,1]

[1] *Top Health S.A.P.I de C.V.*

[2] *Tecnologico de Monterrey, School of Medicine and Health Science*

[3] *Tecnologico de Monterrey, School of Engineering and Science*

Corresponding author's email: greciacardo@hotmail.com

Tympanic membrane perforation (TMP) is a common condition in ENT clinics and is generally attributed to trauma, chronic otitis media (COM) or ventilation tubes placement [1]. Although most TMP cases heal spontaneously in a few weeks, chronic perforations can cause permanent alterations in the middle ear or hearing impairment [2]. The current TMP treatment involves the placement of a paper patch upon the perforation, or a surgical procedure (tympanoplasty) whereby a material, such as an autologous tissue (fascia or cartilage) is grafted [3,4]. We produced a human collagen membrane for eardrum regeneration (HCM-E) that reduces the amount of collagen needed, without resorting to more expensive methods. HCM-E is an allogenic graft for TMP treatment, an alternative that will be cost-efficient and will obviate the immunological risks and invasive procedures. The HCM-E is constituted of type I collagen and has a porosity of 83.81 ± 7.09 %. Crosslinking of the graft resulted in improvement of the mechanical properties, and a degradation time of 3.72 ± 0.48 h assessed by an in vitro collagenase digestibility assay. Furthermore, the crosslinking process did not affect the HCM-E's cytocompatibility, as shown by the residual formaldehyde content that was below 0.001% (v/v) [5]. This was later confirmed by seeding human primary fibroblast from skin onto the HCM-E and stained by DAPI and Phalloidine after 10 days in culture. Finally, we observed that the HCM-E supported and promoted cell growth and migration and could be a possible scaffold for eadrum regeneration.

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Abstract **#153**

THE SHAPE-EFFECT OF CALCIUM PHOSPHATE NANOPARTICLES ON THEIR OSTEOGENIC PROPERTIES.

Pichaporn Sutthavas$_P$[1], Pamela Habibovic[1], Sabine Van Rijt[1]

[1] *MERLN, Maastricht University*

Corresponding author's email: p.sutthavas@maastrichtuniversity.nl

Calcium phosphates in the form of hydroxyapatite (HA) have been extensively studied in the context of bone regeneration due to their chemical similarity to natural bone mineral. While HA is known to promote osteogenic differentiation, the structural properties of the ceramic have been shown to affect the extent of this effect; several studies have suggested that nanostructured HA can improve the bioactivity. However, the role shape plays in the osteogenic potential is more elusive. Here we studied the effect of HA nanoparticle shape on the ability to induce osteogenesis in human mesenchymal stromal cells (hMSCs) by developing nanoparticle films using three different nanoparticle-size HA. We showed that the HA films made from all three shapes of nanoparticles induced increased levels of osteogenic markers on protein and gene level in comparison to hMSCs cultured on cover glass slides. However, their expression levels and profiles differed significantly as a function of nanoparticle shape. In addition, we showed that nanoparticle films were more efficient in inducing osteogenic gene expression in hMSCs compared to adding nanoparticles to hMSCs in culture media. Finally, we demonstrated that hMSC morphology upon adhesion to the HA nanoparticle films is dependent on nanoparticle shape. Our data suggests that HA nanoparticle films are efficient in inducing hMSC osteogenesis in basic cell culture conditions and that nanoparticle shape plays a vital role in cell adhesion and morphology and extent of induction of osteogenic differentiation.

Abstract **#155**

A NEW APPROACH FOR FABRICATING ARTIFICIAL TESTIS

zahra bashiri[P][1,2], Morteza koruji[1,2], Iraj Amiri[3], Mazaher Gholipourmalekabadi[4,2], Reza Falak[5], Hamidreza Asgari[1,2]

[1] Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

[2] Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.

[3] Research Center for Molecular Medicine, Hamadan University of Medical Sciences, 65178 Hamadan, Iran.

[4] Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.

[5] Immunology Research Center (IRC), Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran

Corresponding author's email: zbashiri88@gmail.com

Background: Spermatogonial stem cell (SSC) loss due to cancer treatment, developmental disorder, or genetic abnormality may cause permanent infertility. Tissue and cell culture, 3D scaffolds, and adding of supplements recently have created a new perspective for the differentiation of stem cells in vitro. Therefore, the purpose of this study was to use sheep testicular extracellular matrix (T-ECM) as a biological material for the culture of mouse SSCs in vitro.Materials and Methods: The extracted T-ECM (with different percentages of 0, 1.5, 3, 5%) was used to print the hydrogel scaffold with alginate-gelatin. After cross-linking using aqueous CaCl2 + glutaraldehyde solution, mechanical tests were performed to evaluate the compressive strength and FTIR, degradability, and swelling tests to evaluate the structural and biological properties of the scaffolds. The surface morphology of the scaffold was examined using SEM. Non-toxicity and scaffolds cell adhesion for SSCs was studied using MTT assay and SEM. Result: Results of our study showed that increasing the concentration of ECM in printed scaffolds could increase the hydrophilicity of the scaffold and subsequently enhance the swelling properties that in the printed scaffolds with 5% ECM was acceptable. Also, the results of SEM and MTT assay demonstrated that the printed scaffolds with 5% ECM had proper surface properties, cell adhesion, and good biocompatibility than other groups. Conclusion: In general, our study suggested that T-ECM can be used as a bio-ink for design a functional bioartificial testis in vitro that would offer new fertility restoration options.

Keywords

3D Printing; Artificial Testis; Testicular Extracellular Matrix

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Abstract **#156**

3D IN VITRO MODELS OF EARLY AND LATE HUMAN CARDIAC FIBROSIS BY TUNING 3D ARCHITECTURE OF BIOARTIFICIAL SCAFFOLDS

Mattia Spedicati[1,2,3], Irene Carmagnola[1,2,3], Alice Zoso[1,2,3], Gerardina Ruocco[1,2,3], Valeria Chiono[1,2,3]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *POLITO Biomedlab, Politecnico di Torino, Turin, Italy*

[3] *Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Italy.*

Corresponding author's email: mattia.spedicati@gmail.com

Myocardial infarction causes cardiomyocytes loss, and extracellular matrix (ECM) remodelling, resulting in the progressive formation of stiff dysfunctional fibrotic tissue mainly populated by cardiac fibroblasts1. In vitro disease models of human cardiac tissue reproducing post-infarct microenvironment at different stages could improve preclinical validation of new therapies for heart regeneration, in agreement with the 3Rs principle.

Adult human cardiac fibroblasts (AHCFs) were cultured on 3D polycaprolactone (PCL) scaffolds fabricated by melt-extrusion additive manufacturing and surface grafted with type A gelatin (G), to engineer in vitro early and late stage cardiac fibrosis, depending on scaffold architecture. Early stage fibrosis was reproduced on scaffolds with square grid-shaped meshes and different pore sizes (150 µm; 350 µm), while late-stage fibrosis was reproduced on scaffolds with rectangular grid-shaped meshes (300x150 µm2 pore area). G was efficiently grafted through polyDOPA pre-coating as confirmed by QCM-D (performed both on bare and PCL coated gold sensor), ATR-FTIR, AFM, XPS, static contact angle analysis and profilometry. G coating stability was demonstrated by sample incubation in physiological-like conditions. After culturing AHCFs for 3 weeks and subsequent decellularisation, ECM decorated scaffolds were analysed by immunostaining and Western Blot. Activation of AHCFs was studied based on α-smooth muscle actinin (α-SMA) expression.

As a conclusion, long-term AHCFs cultures on bioartificial scaffolds allowed reproducing the progression of human cardiac fibrosis depending on scaffold architectures (orientation and pore size).

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Abstract **#157**

USING STRUCTURAL MICROHETEROGENEITIES TO CONTROL THE MACROELASTICITY OF BIOINKS AND THUS PRINTING QUALITY AND CELL VIABILITY IN 3D BIOPRINTING

Bruna Maciel[P][1], Claude Oelschlaeger[1], Norbert Willenbacher[1]

[1] *Karlsruhe Institute of Technology (KIT), Institute for Mechanical Process Engineering and Mechanics, Gotthard-Franz-Str.3, 76131 Karlsruhe, Germany*

Corresponding author's email: bruna.maciel@kit.edu

For successful 3D bioprinting, it is well known that bioink rheological properties namely viscosity, yield stress and shear-thinning have to be considered as they can all affect the printing quality and cell viability.
Here we investigated an aspect which has not been considered so far, namely the micro-heterogeneity of the bioink and we established effects of hydrogel structural micro-heterogeneities on the macroelasticity as well as on printing quality and cell viability. Classical rotational rheometry and multiple particle tracking (MPT) based microrheology have been used to characterize macro- as well as micromechanical and -structural properties, respectively. Bioinks of different composition have been investigated: pure alginate and gelatine solutions; alginate (pre-) crosslinked with calcium salts; mixtures of alginate/poly(vinyl alcohol) (PVA), gelatine/PVA and gelatine/nanosilicate clay (Laponite). Pure polymer solutions show a homogeneous microstructure independent of the solvent used. In contrast, for (pre-) crosslinked gels and mixtures, measurements reveal a heterogeneous structure on the micrometer-scale with formation of viscous inclusions within a highly elastic matrix in both water and in cell culture medium. Viscosity, size and spatial distribution of these inclusions have been characterized. Additionally, we observed that gels with the higher degree of heterogeneity exhibit a higher macro elasticity. Finally, hydrogels have been 3D-printed and a correlation between microheterogeneities, cell viability and printing quality has been established. These results will help to better understand the relationship between the bioink´s microstructure before printing and cell viability after printing, as well as feature size and shape fidelity of 3D printed objects.

Keywords

Microrheology; Hydrogels; Microheterogeneity

Abstract **#159**

OSTEOGENIC ACTIVITY OF ACTIFUSE™ IS NOT ENHANCED BY PRE-ADSORBED BMP2

Jasmin Kohnken-Sawall$_P$[1], Simon C. F. Rawlinson[2], Karin A. Hing[1]

[1] *School of Engineering and Materials Science, Queen Mary University of London, London, United Kingdom*

[2] *Institute of Dentistry, Barts & The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom*

Corresponding author's email: j.kohnken-sawall@qmul.ac.uk

BMP2 has a greater affinity for silicate-substituted hydroxyapatite (SiHA) than stoichiometric HA [1], leading to the hypothesis that the enhanced osteoinductivity of SiHA bone graft substitutes (BGS) is associated with surface enrichment of BMP2. We assessed the proliferation and osteogenic differentiation of human mesenchymal stem cells (hMSCs) on SiHA-BGS with and without BMP2.

hMSC were cultured on 80% porous 0.8wt% SiHA-BGS granules (Actifuse™, Baxter Inc, AF) with pre-adsorbed BMP2 (AF+BMP2) or without (AF) for up to 21 days. hMSCs were cultured on plastic in the absence of both AF and BMP2 (control) or in the presence of 300ng BMP2 (control+BMP2). Osteogenic differentiation was assessed by real-time RT-qPCR of bone markers, analysis of ALP activity, and procollagen-1 production.

From Days 3-21, DNA was significantly higher on AF than on AF+BMP2. Control+BMP2 promoted similar specific ALP activities compared with control, whereas AF+BMP2 resulted in higher specific ALP activity over 21 days. hMSCs on AF demonstrated significantly greater specific ALP activity compared to all samples. AF and AF+BMP2 significantly stimulated procollagen production from Days 7-21 compared to control and control+BMP2. control+BMP2 generated the highest osterix expression, suggesting a stronger osteogenic effect of BMP2 in solution. The opposite was found for endogenous BMP2 expression: AF+BMP2 promoted the significantly highest expression from Days 1-21.

Incubation on AF supported osteogenic differentiation of hMSCs compared with control. AF+BMP2 and control+BMP2 significantly stimulated early osteogenic gene expression, but it did not translate into increased osteogenic behaviour. Enhanced SiHA-BGS osteoinductivity might not be linked to surface enrichment of BMP2.

Keywords

BMP-2; Mesenchymal stem cells; Bone regeneration

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Abstract **#160**

THE ETHICAL IMPLICATIONS OF TISSUE ENGINEERING FOR REGENERATIVE PURPOSES: A SYSTEMATIC REVIEW

Anne-Floor de Kanter[P][1], Karin Jongsma[1], Annelien Bredenoord[1]
[1] *Department of Medical Humanities, Julius Center, University Medical Center Utrecht, Utrecht, The Netherlands*
Corresponding author's email: a.j.dekanter@umcutrecht.nl

It has been argued that tissue engineering (TE) is a paradigm-changing and potentially disruptive technology (1,2). The ethical and societal implications of TE therefore need to be mapped out and evaluated in parallel to its development (3). To this end, we have systematically reviewed the ethical implications of the development and application of tissue engineering for regenerative purposes, as mentioned in the academic literature. Our review includes over 200 articles published in the period 2008-2020. The major results of this review will be presented in this paper.

A number of recurring themes emerge from our review. These include the need to balance hype and hope in media representations of TE and Regenerative Medicine (RM), the question of when a TE technology is safe enough to enter clinical translation, and the challenge of reducing, replacing and refining animal experimentation during TE development. In this paper we will highlight which ethical aspects of TE have received remarkably little attention to date. Notably, this includes ethical questions related both to the earliest and the latest phases of technology development, i.e. research preceding the involvement of animal and human subjects, and future outlooks on the (societal) implications of TE on the long term.

Overall, through our systematic review, we hope to provide insight into the ethical and societal implications of TE, to serve as a practical guide to researchers working in this field, and as a starting point for further exploration of as yet underexplored ethical implications of TE.

Keywords
tissue engineering; ethics; societal impact

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Abstract **#162**

THE MECHANICAL CONTROL OF CYTOSKELETON DYNAMICS BY YAP-TEAD1 DRIVES HUMAN PLURIPOTENT STEM CELL MESODERM SPECIFICATION

Stefania Pagliari[1,2], Vladimir Vinarsky[1,2], Fabiana Martino[1,2,3], Ana Rubina Perestrelo[1,2], Jorge Oliver De La Cruz[1,2], Guido Caluori[1], Jan Vrbsky[1,2], Giancarlo Forte[1,2]

[1] *International Clinical Research Center (ICRC) of St Anne's University Hospital (FNUSA)*

[2] *Competence Center for Mechanobiology in Regenerative Medicine, INTERREG ATCZ133*

[3] *Faculty of Medicine, Department of Biology, Masaryk University*

Corresponding author's email: stefania.pagliari@fnusa.cz

The fine-tuning of cytoskeleton dynamics is crucial for a number of cellular processes, including migration, division and differentiation and is guided by a complex interplay between biochemical and mechanical cues arising from the extracellular matrix (ECM) or provided by the neighbouring cells. YAP-TEAD respond to cell-cell interaction and to substrate mechanics and, among their downstream effects, prompt focal adhesion (FA) gene transcription, thus contributing to FA-cytoskeleton stability. This activity is key to the definition of adult cell mechanical properties and function. The mechanobiology of pluripotent stem cells (PSCs) is still poorly understood.

Here we provide evidence that human PSCs display a sustained basal YAP-driven transcriptional activity despite they grow in very dense colonies, indicating these cells are insensitive to contact inhibition. PSC inability to perceive cell-cell interactions can be restored by tampering with Tankyrase enzyme, thus favouring AMOT inhibition of YAP function. YAP-TEAD complex is promptly inactivated when germ layers are specified, and this event is needed to adjust PSC mechanical properties in response to physiological substrate stiffness. By providing evidence that the mechanical activation of YAP-TEAD1 complex affects the expression of a handful of genes encoding for proteins involved in cytoskeleton dynamics, we suggest that substrate mechanics can direct PSC specification by influencing cytoskeleton arrangement and intracellular tension. We propose an aberrant activation of YAP-TEAD1 axis alters PSC potency by inhibiting cytoskeleton dynamics, thus paralyzing the changes in shape requested for the acquisition of the given phenotype.

KEYWORDS

Pluripotent stem cells, cytoskeleton, cell mechanobiology

Abstract **#163**

MODELLING EARLY-TO-LATE STAGE PROGRESSION OF CARDIAC FIBROSIS THROUGH "BIOARTIFICIAL" SCAFFOLDS: AN IN VITRO PLATFORM FOR PRECLINICAL VALIDATION OF NEW THERAPIES IN CARDIAC DISEASE-MICROENVIRONMENTS

Alice Zoso[P][1,2,3], Irene Carmagnola[1,2,3], Mattia Spedicati[1,2,3], Gerardina Ruocco[1,2,3], Marcello Manfredi[4], Franca Di Meglio[5], Clotilde Castaldo[5], Daria Nurzynska[5], Valeria Chiono[1,2,3]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *POLITO Biomedlab, Politecnico di Torino, Turin, Italy*

[3] *Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Italy*

[4] *Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy*

[5] *Department of Public Health, University of Naples Federico II, Naples, Italy*

Corresponding author's email: alice.zoso@polito.it

Myocardial infarction causes a deep remodelling of cardiac ECM and progressive formation of stiff fibrotic tissue, populated by cardiac fibroblasts[1]. In vitro models of post-infarct microenvironment are required for preclinical validation of new therapies for myocardial regeneration. However, cardiac fibrotic tissue is characterised by high variability in terms of composition, size, stiffness and structure, depending on location and time from heart attack. The aim of this work was to design a platform of 2D and 3D "bioartificial" scaffolds, based on polycaprolactone (PCL) and gelatin (G), fabricated by different techniques (electrospinning and melt-extrusion additive manufacturing) with tuned architectures to mimic the variety and progression of cardiac fibrosis.

Adult human cardiac fibroblasts from both healthy and pathological human ventricle (HCFs) were cultured on scaffolds for 3 weeks. Upon scaffold decellularization, deposition of the typical cardiac ECM proteins by cells was evaluated by immunofluorescence, immunoblot and proteomics analyses as a function of scaffold architecture and healthy and pathological HCFs. Scaffold architecture affected stiffness and porosity, which in turn influenced the type of cell-secreted proteins, their distribution and orientation. Comparisons of engineered constructs with human infarcted samples are in progress to validate the engineered "bioartificial" tissues as platforms for in vitro modelling of cardiac fibrosis progression, tuned by scaffold architecture. Results will be beneficial for the preclinical investigation of new therapies and the study of different degrees of cardiac fibrosis.

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No-772168.

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Abstract **#165**

3D ACTIVE BONE FORMING MODEL TO STUDY AMELOBLASTOMA

Deniz Bakkalci[P][1]

[1] *Researcher*

Corresponding author's email: deniz.bakkalci.16@ucl.ac.uk

Ameloblastoma is a locally aggressive tumour of the jawbones and associated with disruption of bone homeostasis, which is the balance between the removal of mineral and organic constituents of the bone matrix by osteoclasts and the new bone formation by osteoblasts. The precise molecular mechanisms regulating the cross-talking between ameloblastoma tumour cells and the surrounding osteoclasts and osteoblasts remain unknown. We have been working on the development of a biomimetic 3D model of ameloblastoma and its relevant tumour microenvironment, in order to investigate the cellular mechanisms and molecular pathways behind the bone resorption of ameloblastoma. Here, we have managed to establish 3D tumoroid models for two different histological subtypes of ameloblastoma; plexiform and follicular. The models are composed of an artificial cancer mass-composed of either AM-1 cells (plexiform ameloblastoma cell line) or AM-3 (follicular ameloblastoma cell line) and a surrounding bone-like stroma with active bone-forming osteoblasts. The surrounding stroma was developed by incorporating primary osteoblasts from the calvaria of neonatal rats. All cell types were grown in 2D to be used as controls. Our findings show that the tumoroid models present histological features similar to those of classical ameloblastoma from patient samples. We managed to form ~7 times more bone nodules at an earlier day in our 3D active bone forming models compared to 2D control (*$p<0.05$). We also demonstrated that ameloblastoma cells inhibited bone formation in all of our tumoroid models (n=9). Our data first to indicate that, ameloblastoma cells inhibit osteoblast-driven bone nodule formation.

Abstract **#166**

3D PRINTED CELL-LADEN CONSTRUCTS FOR SKELETAL MUSCLE REGENERATION

Sahar Salehi[P][1], Mairon Trujillo-Miranda[2], Claudia Müller[1]

[1] *Department of Biomaterials, Univerisity of Bayreuth*

[2] *University of Bayreuth*

Corresponding author's email: sahar.salehi@bm.uni-bayreuth.de

Highly aligned and dense microbundles composed of fibers are the major portion of the muscles. Nevertheless, the primary source of such hierarchical structure is specialized cells called myoblasts which are able to differentiate and form multinuclear myotubes and finally myofibers [1]. Alignment and direction that such fused cells adopt within the natural constructs are critical for the vitality of the muscle and its proper function [2]. In this study, we aim to study the fabrication of 3D construct for skeletal muscle tissue regeneration and the effect of external stimuli on the formation of muscle fibers from encapsulated muscle cells.

Therefore, we attempt to evaluate a printable bioink comprising of a methacrylated gelatin (GelMA) and photoinitiators with various concentrations and different crosslinking times which can undergo cyclic stretching (Scheme 1). Moreover, this appropriate bioink must support the cell growth, proliferation and differentiation to form organized muscle fibers. Therefore, the rheological behavior of the bioink and mechanical properties of the hydrogels were measured using rheometer and compression test, respectively. Swelling degree was monitored after 24 hr and at last, 3D printed construct was stretched mechanically under various cycles.

It was shown that pre-incubation at 4°C increased the mechanical stability of GelMA hydrogels, and additional photocrosslinking using VA-086 as a photoinitiator, led to production of porous and flexible hydrogels with high compressive modulus and crosslinking density. After 7 days of differentiation encapsulated mouse myoblasts cells (C2C12) in the ink showed significant differences in formation of myofibers with and without mechanical loading.

Keywords

external stimuli

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Abstract **#167**

3D BIOPRINTING OF PERFUSABLE VASCULARIZED TISSUES MODELS OF THE NEUROVASCULAR UNIT

Ying (Betty) Li[P][1], Ewa Baumann[1], Junzhou Huang[1], Caroline Sodja[1], Claudie Charlebois[1], Anna Jezierski[1]

[1] *National Research Council Canada*

Corresponding author's email: Betty.Li@nrc-cnrc.gc.ca

Recent advances in 3D microfluidic based bioprinting have enabled manufacturing of complex structures with controlled architectures and tunable properties. Coupling 3D bioprinting with cells derived from human induced pluripotent stem cells (iPSCs), represents a unique opportunity towards modeling complex tissues in an effort to de-risk preclinical drug discovery pipelines. In this study, we use a novel multi-layered flow focusing printhead and validated bioinks compatible with bioprinting iPSC-derived brain endothelial cells (iBECs) to bioprint multi-cellular in vitro perfusable blood vessel models, consisting of a continuous iBECs surrounded by i-neurons and i-astrocytes that make up the neurovascular unit. For each cell type, the effects of different bioink composition, composed of alginate functionalized with gelatin, collagen, fibronectin and laminin as well as extracellular matrix (ECM) remodeling will be investigated and correlated to material biocompatibility by evaluating cell attachment, viability, growth and collagen deposition. The 3D bioprinted construct will be incorporated into a microfluidic device platform to facilitate perfusion and cell maturation while enabling controlled dosing and sampling of test compounds to assess drug efficacy. The ability to print iPSC cells and perfusable blood vessels within 3D bioprinted tissue constructs holds great potential for the creation of more physiologically relevant in vitro models suitable especially as platforms for the pre-screening of drugs, such as the blood brain barrier.

Keywords

3D bioprinting; blood-brain barrier; vascularization

Abstract **#169**

OSTEOINDUCTIVE AND ROS SCAVENGING EXTRACELLULAR MATRIX MIMETIC MINERAL FIBERS FOR BONE REGENERATION

Hayeon Byun[P][1], Heungsoo Shin[1]

[1] *Hanyang University*

Corresponding author's email: leodenter@hanyang.ac.kr

Biomineralization in body is essential in bone regeneration due to its osteoinductive and osteoconductive properties. Thus, there have been several methods to induce biomineral formation on the surface of biomaterials with mimicking its structure and composition. Among them, simulated body fluid (SBF) has been used as a method performed under physiologically relevant temperature. However, this process still has several limitations including long processing time and poor mineralization on unpolarized material surface. Additionally, generation of reactive oxygen species (ROS) and induction of pro-inflammatory genes by the synthetic biominerals have been reported. In this study, we developed a method for multi-functional mineralization method using extracellular matrix (ECM) like synthetic fibers with osteoinductivity and ROS scavenging ability by employing epigallocatechin gallate (EGCG), a polyphenol potentially forming biominerals in SBF via phenol metal network and ROS. We success fully controlled mineralization on the fibers and confirmed hydroxyapatite-like chemical structure of the mineral. To investigate the effect of the biomineralized nanofibers, human adipose-derived stem cells (ADSCs) were assembled with the prepared nanofibers as spheroids, as a module for in vitro analysis and in vivo transplantation. The composite spheroids showed ROS scavenging effect under oxidative stress condition and in vitro osteoinductive activity of ADSCs. Furthermore, transplanted composite spheroids enhanced in vivo bone regeneration in mouse calvarial defect model. In conclusion, we verified rapid and controlled mineralization via our system and multi-functionality related to bone regeneration. Therefore, this novel biomineralization method and advanced spheroid fabrication system could be utilized for bone tissue engineering.

Keywords

Biomineralization; metal-phenolic network; Bone tissue engineering

Abstract **#170**

A NOVEL HYDROGEL-BASED TREATMENT FOR COMPLETE TRANSECTION SPINAL CORD INJURY REPAIR IS DRIVEN BY MICROGLIA/MACROPHAGES REPOPULATION

He Shen[P][1]

[1] *Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences*

Corresponding author's email: clover_4_hope@sina.com

Microglia/macrophage mediated-inflammation, a main contributor to the microenvironment after spinal cord injury (SCI), persists for a long period of time and inhibits SCI repair. However, the effects of microglia/macrophage mediated-inflammation on neurogenic differentiation of endogenous neural stem/progenitor cells (NSPCs) are not well understood. In this study, to attenuate activated microglia/macrophage mediated-inflammation in the spinal cord of complete transection SCI mice, a combination of photo-crosslinked hydrogel transplantation and CSF1R inhibitor (PLX3397) treatment was used to replace the prolonged, activated microglia/macrophages via cell depletion and repopulation. This combined treatment in SCI mice produced a significant reduction in CD68-positive reactive microglia/macrophages and mRNA levels of pro-inflammatory factors, and a substantial increase in the number of Tuj1-positive neurons in the lesion area compared with single treatment methods. Moreover, most of the newborn Tuj1-positive neurons were confirmed to be generated from endogenous NSPCs using a genetic fate mapping mouse line (Nestin-CreERT2;LSL-tdTomato) that can label and trace NSPC marker-nestin expressing cells and their progenies. Collectively, our findings show that the combined treatment method for inhibiting microglia/macrophage mediated-inflammation promotes endogenous NSPC neurogenesis and improves functional recovery, which provides a promising therapeutic strategy for complete transection SCI.

Abstract **#171**

REGENERATIVE MEDICINE AND ENGINEERING – A NEW BACHELOR PROGRAM

Jurica Bauer$_P$[1], Guy Bendermacher[1], Vanessa LaPointe[1], Pamela Habibović[1]

[1] *Maastricht University*

Corresponding author's email: j.bauer@maastrichtuniversity.nl

Regenerative Medicine is a relatively new field at the intersection of science, engineering and medicine. Traditionally, researchers in this field are biologists, chemists, materials scientists, data scientists, engineers or physicians who have acquired skills and knowledge beyond their basic training and stepped into the multidisciplinary world of regenerative medicine. Many of them, however, still approach their research questions largely from the perspective of their own discipline and find it difficult to step outside that domain. Having recognized the need for a new multidisciplinary researcher profile that would receive basic training in all the required disciplines to easily enter the aforementioned field, we have taken the initiative of designing an undergraduate program focusing on Regenerative Medicine and Engineering. Within this program we aim to educate a new generation of researchers that will swiftly adapt to any area of regenerative medicine and develop medical therapies and products for research and clinical use. Applying the principles of problem- and research-based learning, our program will offer a solid STEM foundation, which will be strongly integrated with medicine. An example of this is a real-world student research project defined by clinicians within the academic hospital. In addition, special attention will be paid to the development of the translational scientific skills necessary to bring new therapies and products onto the market.

Alongside discussing our progress in this endeavor, in this interactive session anyone with an interest in education will be most welcome to share their own vision and experiences relating to education in regenerative medicine.

Keywords

Undergraduate/Bachelor Program; Multidisciplinary; Problem-Based Learning

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Abstract **#172**

MECHANICALLY ROBUST AND ELASTIC TROPOELASTIN-POLYGLYCEROL SEBACATE COMPOSITES AS SMALL-DIAMETER VASCULAR GRAFTS

Ziyu Wang[P1,2], Suzanne Mithieux[1,2], Howard Vindin[1,2], Yiwei Wang[3], Linyang Liu[1,2], Yuchi Matsuzaki[4,5], Tai Yi[4,5], Kevin Blum[4,5], Nguyen Pham[6,7], Brian Hawkett[6,7], Toshiharu Shinoka[4,8], Christopher Breuer[4,9], Anthony Weiss[1,2,7]

[1] *School of Life and Environmental Sciences, University of Sydney, NSW, Australia*

[2] *Charles Perkins Centre, University of Sydney, NSW, Australia*

[3] *Anzac Research Institute, NSW, Australia*

[4] *Center for Regenerative Medicine, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, USA*

[5] *Department of Biomedical Engineering, Ohio State University, Columbus, OH, USA*

[6] *Key Centre for Polymers and Colloids, School of Chemistry, The University of Sydney, NSW, Australia*

[7] *University of Sydney Nano Institute, The University of Sydney, NSW, Australia*

[8] *Department of Cardiothoracic Surgery, The Heart Center, Nationwide Children's Hospital, Columbus, OH, USA*

[9] *Department of Surgery, Ohio State University Wexner Medical Center, Columbus, OH, USA*

Corresponding author's email: zwan6054@uni.sydney.edu.au

Compromised arteries such as those suffering from severe atherosclerosis can lead to myocardial infarction, which is the leading cause of death worldwide. Autologous or commercially available synthetic grafts can be used as artery replacement, but these often fail to provide long-term patency and display limited regeneration. There is a high demand for alternative grafts that can develop into neovessels, yet do not rupture or occlude after surgery. Towards this goal, we have fabricated vascular grafts from a combination of tropoelastin (TE), the soluble precursor to elastin, and polyglycerol sebacate (PGS), a highly elastic degradable biomaterial.

Scaffolds were fabricated into a spectrum of microstructures from TE fiber-reinforced PGS matrix to pervasive TE-PGS fiber-networks, resulting in scaffolds with mechanical properties comparable to the human coronary artery. The addition of TE promoted the attachment and proliferation of organized vascular endothelial and smooth muscle cells. Vascular endothelial cells formed a monolayer in vitro and expressed functional markers of endothelium including VE-Cad, eNOS, and vWF. Vascular smooth muscle cells changed from rhomboid to spindle shapes on scaffolds and expressed contractile phenotype markers such as alpha-SMA and MHC II, which have been shown to contribute to reducing the risk of hyperplasia. Implantation in mice aorta showed the compliance of the patent TE-PGS vascular grafts continued to increase in 6 weeks. Hematoxylin and eosin stain showed the graft is being degraded by immune cells. Verhoeff-Van Gieson stain showed de novo regeneration of elastic fibers and their organization into long and continuous elastin similar to native elastic laminae.

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Abstract **#173**

THE SURVEY ON CELLULAR AND TISSUE-ENGINEERED THERAPIES IN EUROPE IN 2016 AND 2017

Max Gay$_P$[1], Helen Baldomero[2], Dominique Farge-Bance[3], Pamela G. Robe[4], Scott Rodeo[5], Jakob Passweg[2], Magdalena Müller-Gerbl[1], Ivan Martin[6]

[1] *Institute of Anatomy, Department of Biomedicine, University of Basel, Switzerland*

[2] *EBMT Activity Survey Office, Division of Hematology, University Hospital Basel, University of Basel, Basel, Switzerland*

[3] *Unité de Médecine Interne: Maladies Auto-immunes et Pathologie Vasculaire, Université de Paris; MATHEC, Centre de Référence des Maladies auto-immunes systémiques Rares d'Ile-de-France, Hôpital St-Louis, Paris, France*

[4] *National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA*

[5] *Orthopaedic Surgery, Weill Medical College of Cornell University; The Hospital for Special Surgery, New York, NY, USA*

[6] *Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland*

Corresponding author's email: max.g@unibas.ch

This report describes activity in Europe for the years 2016 and 2017 in the area of cellular and tissue-engineered therapies, excluding hematopoietic stem cell treatments for the reconstitution of hematopoiesis. It is the eighth of its kind and is supported by five established scientific organizations amongst them TERMIS-EU. In 2016 and 2017, a combined 234 teams from 29 countries responded to the survey; 227 teams reported treating 8236 patients in these two years. Indications were categorized in hematology/oncology (40%; predominantly prevention or treatment of graft versus host disease and hematopoietic graft enhancement), musculoskeletal/rheumatological disorders (29%), cardiovascular disorders (6%), neurological disorders (4%), gastrointestinal disorders (<1%), as well as miscellaneous disorders (20%), which were not assigned to the previous indications. The predominantly used cells were autologous (61%). The majority of autologous cells were used to treat musculoskeletal/rheumatological (44%) disorders, whereas allogeneic cells were mainly used for hematology/oncology (78%). The reported cell types were mesenchymal stem/stromal cells (MSCs) (56%), hematopoietic cells (21%), keratinocytes (7%), chondrocytes (6%) dermal fibroblasts (4%), dendritic cells (2%), and other cell types (4%). Cells were expanded in vitro in 62% of the treatments, sorted in 11% of the cases, and rarely transduced (2%). The processing of cells was out-sourced to external facilities in 30% of the cases. Cells were delivered predominantly intravenously or intra-arterially [47%], as suspension [36%], or using a membrane/scaffold (16%). The surveying of cell and tissue-engineered therapies is a relevant instrument to capture developing trends and monitor advances in the field, based on effective treatments performed.

Abstract **#174**

IMPROVING THE MIGRATION OF INTRAVENOUSLY INJECTED MESENCHYMAL STEM CELLS TO INFLAMMATION SITES BY CELL SURFACE ENGINEERING WITH POLYETHYLENE GLYCOL

Yukiya Takayama[P][1], Kosuke Kusamori[1], Makiya Nishikawa[1]

[1] *Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Tokyo University of Science*

Corresponding author's email: 3a17702@ed.tus.ac.jp

Mesenchymal stem cells (MSCs) have some notable functions such as homing to inflammation sites, tissue repair, and immune regulation. However, the homing rate of intravenously injected MSCs to inflammation sites is as low as less than 1% due mainly to their entrapment in the lung vasculature. We previously reported that cell surface modification using the avidin-biotin complex (ABC) method was useful to stably modify MSCs with various biotinylated compounds.1 In this study, we attempted to modify the surface of MSCs with polyethylene glycol (PEG), a biocompatible polymer, using the ABC method to avoid the lung entrapment after intravenous injection through inhibition of the intercellular adhesion between MSCs and vascular endothelial cells in the lung. PEG-modified MSCs were prepared by sequentially reacting MSCs with sulfo-NHS-LC-biotin, streptavidin and PEG-biotin. PEG-modified MSCs were then seeded onto the confluent monolayered mouse aortic endothelial cells (MAECs). The number of PEG-modified MSCs adhering to MAECs was significantly lower than that of unmodified MSCs. Then, PEG-modified MSCs were intravenously injected to the tail veins of carbon tetrachloride-induced acute liver failure model mice. At 1 h after injection, the number of PEG-modified MSCs in the lung was significantly lower than that of unmodified MSCs. Moreover, significantly more PEG-modified MSCs were observed in the liver than unmodified MSCs at 24 h after injection. Taken together, these results indicate that PEG modification to the surface of MSCs by the ABC method reduces the lung entrapment of MSCs and improves their migration to the inflammation sites after intravenous injection.

Keywords

Cell surface modification; Mesenchymal stem cells; Polyethylene glycol

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Abstract **#175**

BIOCOMPATIBLE, INJECTABLE, IN SITU GELLING TUNICATE NANOCELLULOSE HYDROGELS FOR TISSUE AND ORGAN REPAIR

Eric Thompson[1], Sanna Sämfors[2], Hans Gude Gudesen[1], Christofer Troedsson[1], Paul Gatenholm[2,3]

[1] *Ocean TuniCell AS, Norway*

[2] *3D Bioprinting Center, Chalmers University of Technology, Sweden*

[3] *CELLHEAL, Norway*

Corresponding author's email: eric.thompson@oceantunicell.com

Injectable hydrogels are emerging biomaterials because they provide new avenues to reduce healing time and decrease risks of post-operative infections. Here, we report development of a new generation of injectable, tunable, hydrogels based on cellulose nanofibrils (TUNICELL) isolated from tunicates grown in Norwegian fjords. Tuning is achieved through mechanical and chemical modifications. Processing in a GMP compliant clean room resulted in pure (99% purity), highly crystalline (89%) cellulose fibrils <10 nm in diameter, with tunable lengths. TUNICELL is sterilized, and has endotoxin levels below 0.5 EU/g, meeting medical requirements. Cellulose nanofibril dispersions in water, adjusted from 2 to 4% dry matter, showed very good shear thinning properties as determined by rheological evaluation. Mechanical treatment by passing dispersions through a microfluidizer, allowed control of both fibril lengths (hundreds of nm to several μm), and fibril length distributions, as determined by AFM. We studied injectability using simulated tissues of various stiffness. Shorter fibrils with broader fibril length distributions, and increased concentration of fibrils, improved injectability. Chemical modification to generate carboxymethylated cellulose nanofibrils, which could be crosslinked with calcium ions, resulted in hydrogels with higher shear modulus than crosslinked alginate with the same polymer content. Tunicate nanocellulose hydrogels of varying compositions were evaluated for controlled rates of hemoglobin delivery for wound healing applications, as well as stem cell delivery for cartilage repair.

Abstract **#176**

PREDICTION OF CELL INJURY IN BIOPRINTING PROCESSES

Edwin-Joffrey Courtial[1], Aravind Anandan[P1], Emma Petiot[1], Christophe Marquette[1]

[1] 3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, Bat. Lederer, 1 rue Victor Grignard, 69100 Villeurbanne, FRANCE.

Corresponding author's email: aravind.anandan@univ-lyon1.fr

Bioprinting is one of the most advance technology in tissue engineering and regenerative medicine. Trough LDM (liquid deposition modelling) and ink-jet technics, biomaterials including cells are used to produce tissues with cell density and shape recapitulating human tissue behaviours. However, these processes present limitations such as cells viability, due to a high shear stress value inside the dispensing system. In this study, we evaluated the shear stress impact on the quantification (lysis) and qualification (necrosis, apoptosis) of cells to ensure the production of organoids. Our approach was based on the relation between biomaterial viscoelastic properties, sizing of deposition system and cells viability. To access the shear stress map inside the bioprinting system, a specific algorithm was developed based on Poiseuille tube flow of a pseudoplastic power law fluid. Different kind of cells were used to understand the capacity of them to withstand stress. Living and labelled necrotic or apoptotic cells were counted before and post-printing process to evaluated cell viability and total cell recovery in various conditions. In view of results, the shear stress gradient can be controlled through biomaterial rheological behaviour and sizing of dispensing system. Cells viability seems to be depend on shear stress value but also duration. In future, the recording of data in a library will allow biomaterial engineers to use algorithm in reverse engineering mode, to tailored bioprinting system and/or biomaterials properties in function of the kind of cells. A disruptive innovation could be applied in autograft technique to ensure the success of surgical intervention.

Keywords

Bioprinting; Cells viability; Prediction

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Abstract **#177**

BIOCOMPATIBLE POLYMERIC BLEND WITH ENHANCED MECHANICAL PROPERTIES AND TAILORED DEGRADATION RATE FOR TENDON REGENERATION

Behzad Shiroud Heidari[1,2,3], Peilin Chen[4], Ruan Rui[4], Hani Al-Salami[5], Elena De Juan Pardo[1,2,6], Minghao Zheng[4,7], Barry Doyle[1,2,3]

[1] Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands and Centre for Medical Research, The University of Western Australia, Perth, Australia.

[2] School of Engineering, The University of Western Australia, Perth, Australia.

[3] Australian Research Council Centre for Personalised Therapeutics Technologies, Australia.

[4] Centre for Orthopaedic Research, Faculty of Health and Medical Sciences, The University of Western Australia, Perth, Australia.

[5] Biotechnology and Drug Development Research Laboratory, School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia. Australia.

[6] Science and Engineering Faculty, Queensland University of Technology, Brisbane, Queensland, Australia.

[7] Perron Institute for Neurological and Translational Science, Nedlands, Western Australia, Australia.

Corresponding author's email: behzad.shiroudheidari@research.uwa.edu.au

There is often a trade-off between mechanical properties and degradation rate of biodegradable materials for in vivo applications; hence, finding the right balance in properties for biomedical applications is challenging. In this study, we hypothesise that blending semicrystalline poly (p-dioxanone) (PDO) and poly (lactide-co-caprolactone) (LCL) in a specific composition will improve the toughness while also enabling tailored degradation rate. Hence, blends of semicrystalline PDO and poly (lactide-co-caprolactone) (LCL) were prepared in varying concentrations and formed into films by solvent casting. A range of experimental methods were used to characterize the properties of the blends during hydrolytic degradation in neutral phosphate-buffered saline (PBS) solution at 37 °C for up to 24 weeks. Cellular performance was determined by seeding mouse fibroblasts onto the samples and culturing for 72 hours, before using proliferation assays and confocal imaging. All blends exhibited good cell viability. However, an increase in LCL content causes a reduction in hydrolytic degradation, indicated by weight loss, induced crystallinity, surface and bulk erosions, and tensile properties. A blend of 30% PDO and 70% LCL (PDO3LCL7) resulted in small PDO droplets uniformly dispersed within the LCL matrix and showed toughening behaviour with a notable strain-hardening effect reaching 320% elongation at failure; over 3 times the final elongation of neat LCL, and has an ultimate tensile strength (18.9 MPa) comparable to neat PDO (20.5 MPa). This work highlights that the combination of elastomeric behaviour capable of high deformation, together with moderate degradation rates, particularly suit tissue engineering applications in tendon tissue repair.

Abstract **#183**

MECHANICAL STIMULATION OF FRACTAL BASED SCAFFOLDS TO STEER STEM CELL ACTIVITY

Kenny Van Kampen[P][1], Carlos Mota[1], Lorenzo Moroni[1]

[1] *Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University*

Corresponding author's email: k.vankampen@maastrichtuniversity.nl

Current additive manufacturing (AM) methods such as fused deposition modeling and bioprinting follow a certain tool pathway to deposit material, often referred to G-code. G-code is based on an arranged list of X, Y and Z coordinates that are followed by the printhead to deposit material and is generated from a 3D CAD model into layers. Each layer can have a specific pattern for the print head to follow. However, most automated pattern generation software have limited line patterns that run parallel or circumferential to each other resulting in woodpile structures. Here, we present an automated method for producing a variety of patterns based on fractals. These unexplored patterns have a different mechanical behavior than conventional patterns, such as negative Poisson's ratios or multiple linear elastic regions. In addition, the mechanical stimulation of the fractal scaffolds on induced pluripotent stem cells (iPSCs) pluripotency was assessed.

Scripts were written in Rhino 6 Grasshopper to generate fractal patterns into G-code compatible for AM (Bioscaffolder SYSENG) using Polycaprolactone. The resulting scaffolds were mechanically tested (TA Electroforce 3200). A finite-element model of the scaffolds was used (COMSOL multiphysics) to simulate the stress and displacement. A bioreactor was developed to mechanical stimulate the scaffolds during culture with iPSCs.

The scripts successfully generated different fractal patterns. Compared to a woodpile structure, certain fractals showed multiple linear elastic regions during compression tests or a negative Poisson's ratio during tensile tests. Initial cell culture in static conditions showed that iPSCs did not lose pluripotency on the fabricated scaffolds.

Keywords

Biofabrication; Mechanical stimulation; Induced Pluripotent Stem Cells

Abstract **#186**

MEDICAL ELECTROSPINNING; CHALLENGES AND SOLUTIONS TO BRING FIBER BASED PRODUCTS TO THE MARKET

Judith Heikoop[P][1], Marc Simonet[1], Kikki de Laat[P]

[1] *IME Medical Electrospinning*

Corresponding author's email: m.simonet@ime-electrospinning.com

Fiber-based scaffolds can be a powerful tool to engineer living tissue replacements by providing mechanical, structural and organizational support to guide the development of well-organized and functional extracellular matrices (ECM). In the regenerative medicine field, electrospinning is often the preferred scaffold production technique due to its capability to produce 3-dimensional fibrous ECM-lookalike scaffolds with similar nanometer to micrometer length scales utilizing an extensive range of natural and synthetic polymers. The electrospinning process is highly versatile, allowing to tailor scaffold properties to fit the regenerative demands of various tissues to be engineered.

Controlling all these parameters, defining this method's versatility, has proven to be a challenge, leading to production inconsistencies, batch-to-batch variability and reproducibility issues. Hence, it's of outmost importance to tightly control and log all relevant parameters. For example, the humidity affects the structural properties of the scaffold on the macro and micro level. These structural changes can have a large effect on the mechanical and biological properties of the scaffolds.

Keeping a tight control of the entire production process, combined with real time scaffold thickness monitoring, is an innovative approach to drastically reduce variability of fiber-based scaffolds between and within batches. This also minimizes waste during the production and is therefore a prerequisite for scaling a research process to an economically viable manufacturing process.

Tightly controlled Medical Electrospinning will allow the translation of promising research outcomes into the design and development of scaffolds to promote regeneration of the natural structures of the extracellular matrix in a variety of tissues.

Abstract **#187**

NEW IMMUNE-MODULATORY SURFACES BASED ON COATINGS WITH GLYCOSAMINOGLYCANS

Thomas Groth[P][1]

[1] *Department Biomedical Materials, Institute of Pharmacy, Martin Luther University Halle-Wittenberg*

Corresponding author's email: thomas.groth@pharmazie.uni-halle.de

Implantation of biomaterials triggers inflammation that can be transient defeating infections and promoting healing, or longer lasting undesired chronic, with foreign body response and fibrous encapsulation. Hence, design of biomaterials that control inflammation is desirable. Macrophages represent key players at the implantation site that secrete cytokines and other mediators of inflammation, which synthesis is regulated by nuclear transcription factor-κB (NF-κB) signaling pathways. Glycosaminoglycans (GAG) like heparin (Hep) and hyaluronic acid (HA) were studied here as biomaterial coatings to control inflammation by their effects on human macrophages in vitro and after subcutaneous implantation in mice. GAG were either covalently immobilized or physically adsorbed as multilayers (in combination with chitosan) on model substrata or biphasic calcium phosphate (BCP) particles. In vitro studies on model surfaces could demonstrate reduction in macrophage adhesion, formation of multinucleated giant cells and interleukin-I secretion after immobilization of HA and Hep with heparin being more effective. These effects were strongly related to suppression of NF-kB signaling as studied by immunofluorescence and immunoblotting and related to association and uptake of GAG by macrophages. In vivo studies with mice revealed that coating of BCP particles with HA suppressed the inflammatory response together with formation of new bone-like tissue, while the presence of Hep delayed the onset of inflammation permitting osteogenesis in this subcutaneous bone-forming model. Results of this study may also pave the way to reduction of inflammation after implantation of sensors, soft and hard tissue implants and other medical devices.

Abstract **#188**

CHONDROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS MEDIATED BY EXTRACELLULAR VESICLES FROM PLASMA- AND SERUM-BASED BLOOD-DERIVED PRODUCTS FOR OSTEOARTHRITIS THERAPY

Alexander Otahal[P1], Karina Kramer[1], Olga Kuten-Pella[1,2], Christoph Stotter[1], Zsombor Lacza[2], Stefan Nehrer[1], Andrea De Luna[1]

[1] *Center for Regenerative Medicine, Department for Health Sciences, Medicine and Research, Danube University Krems*

[2] *OrthoSera GmbH*

Corresponding author's email: alexander.otahal@donau-uni.ac.at

Blood-derived products gain increasing interest in the field of regenerative medicine as well as in orthopaedics, aesthetic surgery and cosmetics. Currently, citrate-anti-coagulated platelet-rich plasma (CPRP) preparations are often applied in osteoarthritis (OA), but more physiological and cell-free alternatives such as hyperacute serum (hypACT) are under development. Beside growth factors, blood products also bring along extracellular vesicles (EVs) packed with signal molecules which opens up a new level of complexity at evaluating the functional spectrum of blood products. To attribute potential chondroprotective and anti-inflammatory effects elicited by blood products to EVs, they were enriched from blood products via ultracentrifugation and depletion of frequently co-isolated components such as lipoproteins was verified via Western Blot. EV treatment of primary chondrocytes isolated from OA cartilage enhanced the expression of anabolic markers type II collagen (COL2A1), SRY-box transcription factor 9 (SOX9) and aggrecan (ACAN) compared to full blood products, but also the catabolic marker and tissue remodeling factor matrix metalloproteinase 3 (MMP3), while hypACT EVs prevented increased type I collagen (COL1A1) expression compared to CPRP EVs. CPRP blood product increased SOX9 protein expression, in contrast to hypACT blood product. However, hypACT EVs induced SOX9 protein expression while preventing IL6 secretion. These results indicate that blood EVs are sufficient to induce chondrogenic gene expression changes in OA chondrocytes, while preventing pro-inflammatory cytokine release compared to full blood product. This highlights the potential of blood-derived EVs as regulators of cartilage extracellular matrix metabolism and inflammation as well as candidates for new cell-free therapeutic approaches for OA.

Abstract **#189**

BEYOND THE LIMITS OF DIFFUSION - DEVELOPING NEW HUMANIZED TOOLS TO MODEL DISEASE

Kristina Haase[P][1]

[1] *European Molecular Biology Laboratory*

Corresponding author's email: kristina.haase@embl.es

Complex in vitro models are expected to leap from the bench to the bedside. However, there has been a lack of adoption thus far, resulting from the inability of these models to accurately predict clinical outcomes. Despite advances in patient-specific iPSC and gene-editing technologies, the lack of functional vasculature is hypothesized as a key factor in the limited predictability of current systems. Given its role in regulating oxygen tension, nutrient delivery and immune cell interactions, functional vasculature is required for the development and long-term maintenance of normal tissue physiology. To overcome the limitations of current systems, we develop disease-specific models integrating tissue-specific microvasculature. This talk will provide an overview of several human vascularized models being developed in our lab (placental, ischemic, and tumor). These tools allow us to exploit the self-assembly process of endothelial cells, while tuning the microenvironment to allow for insight into complex tissue biology and pathologic development. These perfusable microvessels enable us to investigate specific cell-cell interactions through live imaging techniques and to understand the effects of mechanical cues, such as luminal flow, on the regulation of microvessel morphology and permeability. In addition, drug dissemination studies can be performed and examined in the context of perfused vascularized tissues. This will lead to further insight into the human response to treatment, and aims to reduce unintended side-effects not seen in animal models. These physiologically relevant microsystems hold significant promise for development into more accurate preclinical models – a major aim of our lab.

Abstract **#191**

NEUROPROTECTIVE AND PRO-ANGIOGENIC 3D STEM CELL SPHEROIDS FOR TREATING ISCHEMIC STROKE

Chieh-Cheng Huang[P][1], Ting-Wei Hsu[1], Yu-Jen Lu[2]

[1] *Institute of Biomedical Engineering, National Tsing Hua University, Hsinchu, Taiwan*

[2] *Department of Neurosurgery, Chang Gung Memorial Hospital, Linkou Medical Center Taoyuan, Taiwan*

Corresponding author's email: chiehcheng@mx.nthu.edu.tw

Ischemic stroke is one of the most frequent causes of death and disability globally. Novel therapeutic strategies for enhancing the repair of neural structures and the recovery of functions are urgently warranted. In this work, we aim to employ a stem cell-based approach for treating ischemic stroke. Three-dimensional (3D) spheroids composed of human mesenchymal stem cells (MSCs), which are capable of secreting neuroprotective agents and inducing therapeutic angiogenesis, and human umbilical vein endothelial cells are constructed using a methylcellulose hydrogel system. Our in vitro results demonstrate that the fabricated 3D stem cell spheroids can protect neurons that are under oxygen glucose deprivation. Additionally, the neurite outgrowth from neurons and tubular network formation by vascular endothelial cells are significantly enhanced. Intracerebral transplantation of 3D stem cell spheroids into the mouse model of middle cerebral artery occlusion effectively reduce lesion area, maintain cerebral structural integrity, and improve post-stroke motor function. In summary, the developed 3D stem cell spheroids possess significant neuroprotective and pro-angiogenic potential, thus holding a great promise to be employed as an effective therapeutic agent for treating ischemic stroke.

Keywords

cell spheroid; mesenchymal stem cells; ischemic stroke

Abstract **#192**

TOWARDS A MATHEMATICAL MODEL OF VASCULAR NETWORK FORMATION IN A HYDROGEL TO AID THE DESIGN AND MANUFACTURE OF ENGINEERED TISSUES

Georgina Al-Badri[1,2], James Phillips[3,2], Rebecca Shipley[4,2], Nick Ovenden[1,2]

[1] *Department of Mathematics, University College London*

[2] *UCL Centre for Nerve Engineering*

[3] *Department of Pharmacology, University College London*

[4] *Department of Mechanical Engineering, University College London*

Corresponding author's email: georgina.kennedy.13@ucl.ac.uk

Vascularisation of implanted engineered tissues is essential to ensure sufficient nutrients to support therapeutic cells once implanted in vivo [1]. Prevascularisation by the inclusion of endothelial cells (ECs), which form capillary-like structures in vitro and anastomose with host vasculature, is one such method [1].

We demonstrate a flexible mathematical model of vascular network formation in a 3D hydrogel, integrating global features such as construct geometry, environmental conditions, and interstitial flow, with key chemical and mechanical cell mechanisms that lead to network formation [2]. Based on a multiphase model framework [3], a set of coupled partial differential equations (PDEs) describe the temporal and spatial evolution of the EC density, extracellular matrix density, oxygen and VEGF concentrations. We explore the impact of key mechanisms including autologous chemotaxis, oxygen-dependent VEGF production, and cell-matrix interactions mediated by traction and matrix stiffness.

Combining data from literature, sensitivity analysis, and parameter optimisation methods, we identify parameter sets under which capillary-like structures form. Using metrics such as vessel length, area, and network complexity, we validate our computational model against in vitro data at the UCL Centre for Nerve Engineering.

Further refinement of the PDE model alongside relevant in vitro experiments will allow us to optimise culture conditions, and elucidate the benefits of interstitial flow towards creating functional prevascularised engineered tissues. The model, implemented and solved in Python, will remain adaptable to hydrogel properties, cell types and culture conditions, with the aim to make the software open for use within other tissue engineering groups.

Keywords

Prevascularisation; Hydrogel; Multiphase model

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Abstract **#194**

PROMOTING SMOOTH MUSCLE CELL MIGRATION IN ELECTROSPUN VASCULAR SCAFFOLDS

Lauren West-Livingston[1,2], Young Min Ju[2], Anthony Atala[2], Sang Jin Lee[2]

[1] *Wake Forest School of Medicine Department of Molecular Medicine and Translational Science*

[2] *Wake Forest Institute for Regenerative Medicine*

Corresponding author's email: lnwest@wakehealth.edu

Objective: This study sought to utilize growth factors to promote the migration of smooth muscle cells (SMC) into an electrospun vascular scaffold. The hypothesis was that the use of growth factors would allow for acellular tissue-engineered vascular grafts that are capable of recruiting smooth muscle cellular proliferation and infiltration in situ.

Methods: Scaffolds electrospun with poly(ε-caprolactone) (PCL)/collagen polymers were surface modified through the passive adsorption of platelet-derived growth factor with two beta subunits (PDGF-BB) and stromal derived growth factor 1α (SDF-1α) at various concentrations, individually and together. Single-factor studies investigated each factor at concentrations of 100 ng/mL, 250 ng/mL, and 500 ng/mL. Dual-factor studies investigated the combined effects of 100 ng/mL PDGF-BB and 500 ng/mL of SDF-1α.

Results: Compared to unmodified controls, scaffolds modified with growth factors showed higher rates of smooth muscle cell proliferation. Notably, scaffolds bioconjugated with a concentration of 100 ng/mL PDGF-BB alone and a concentration of 500 ng/mL SDF-1α alone demonstrated the greatest degree of smooth muscle cell proliferation compared to other single-factor concentrations, which confirmed ideal concentrations reported by other studies. A novel finding was that the combination of these growth factors at the aforementioned concentrations showed synergy in the ability to increase the degree of SMC migration into the depth of the electrospun scaffold.

Conclusion: This series of experiments verifies that growth factors promote proliferation of SMCs on an electrospun scaffold, and that combining multiple factors can optimize migration of SMCs farther into the thickness of electrospun scaffolds in an in vitro setting.

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Abstract **#196**

ADIPOSE TISSUE-DERIVED STROMAL VASCULAR FRACTION SHOWS SUPERIOR OSTEOGENIC DIFFERENTIATION COMPARED TO DONOR-MATCHED MESENCHYMAL STROMAL CELLS

Johanna Husch[P][1], Charlotte Woud[1], Jurriaan Vossen[1], Jeroen van den Beucken[1]

[1] Regenerative Biomaterials - Dentistry, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, The Netherlands

Corresponding author's email: Johanna.Husch@radboudumc.nl

Stromal vascular fraction (SVF) is the primary isolate obtained after enzymatic digestion of adipose tissue that contains various cell types (1). Its successful application for cell-based construct preparation in an intra-operative setting for clinical bone augmentation (2) and regeneration (3) has previously been reported. However, the performance of SVF-based constructs compared to traditional ex vivo expanded adipose tissue-derived MSCs (ATMSCs) remains unclear and direct comparative analyses are scarce (4). Consequently, we here aimed to compare the osteogenic differentiation capacity of donor-matched SVF and ATMSCs. Human adipose tissue from 4 different donors was used to isolate SVF, which was further purified using adherent cell culture to obtain donor-matched ATMSCs. The cell populations were characterized for MSC, endothelial and hematopoietic markers by flow cytometry and immunocytochemistry. For normalized seeding densities of donor-matched cell populations, the plastic adherent fraction within SVF and ATMSCs was determined. Thereafter, SVF and ATMSCs were seeded and cultured in osteogenic differentiation medium for 28 days. Proliferation and mineralization were analyzed during the culture period. ATMSCs showed to be a homogenous cell population, while SVF consisted of different cell lineages. Plastic adherence revealed to be significantly lower for SVF compared to donor-matched ATMSCs. Remarkably, all donor-matched comparisons showed either accelerated or more robust mineralization for SVF which could relate to endothelial cells found present during early osteogenic culture. Our data demonstrate superior osteogenic differentiation capacity of SVF over donor-matched ATMSCs. Further preclinical bone defect studies will explore whether this effect also extends to their performance in bone regenerative applications.

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Abstract **#197**

IMPROVENT OF DECELLULARIZATION PROCESS OF CADAVERIC HOMOGRAFTS IN A NOVEL REACTOR FOR TWO VALVES.

Beni Camacho-Perez[P][1], Octavio Rojas-García[1], Omar Peza-Chávez[1], Fabio González-Sánchez[1], Jesús Granillo Gudiño[1], Juan Pablo Aguilar Aleman[2,1]

[1] *Top Health S.A.P.I de C.V.*

[2] *Tecnologico de Monterrey, School of Engineering and Science*

Corresponding author's email: jp.aguilar@tec.mx

The decellularization of homografts is a process where cells and their waste are removed (>99%) without affecting the extracellular matrix (ECM) while keeping growth factors for cell signalling1. Different methods such as chemical, enzymatic, physical or a combination of them have been reported. The most common involve the use of detergents for solubilization of the cytoplasmic membrane and dissociation of proteins along with the use of nucleases for removal of cellular material. However, these methods may result in an incomplete decellularization, denaturalization of proteins and damage to the ECM1,2.

The aim of this research is to propose and validate a novel process of tissue decellularization that diminishes the damage to the ECM while improving process time and cost. For this, we developed a reactor for using on an aorta and pulmonary artery of a donor, simultaneously. The process consisted of pumping hypotonic solutions in four steps (NaCl ranging from 40 to 0 mM at different time points) for controlling the diffusion and achieving cell lysis with minimal damage to the ECM, digestion with nucleases to remove residual nucleic acids and rinsing with an isotonic solution for remnants removal.

As a result, low amount of residual DNA (< 50 ng of double stranded DNA)2 and not visible nuclear material in histologic analysis with HE staining2 were observed. Cytocompatibility was assessed by exposing the acellular tissue to human fibroblast and adipose derivate human stem cells. We conclude that this could be a potential solution for decellularizing homografts for treating heart congenital defects.

Keywords

Decellularization; Homografts; Reactor

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Abstract **#198**

BIOMATERIAL SURFACE FUNCTIONALIZATION FOR SUSTAINED DELIVERY OF NGF AND BDNF TO STIMULATE NEURITE GROWTH

Ana Sandoval-Castellanos[P][1], John Haycock[1], Frederik Claeyssens[1]

[1] *The University of Sheffield*

Corresponding author's email: amsandovalcastellanos1@sheffield.ac.uk

Peripheral nerve injury is a major cause of disability [1-2]. The clinical standard is the use of autografts; however, their performance is limited. Nerve guide conduits (NGCs) could be used as an alternative. The addition of neurotrophins has been studied to improve the performance of NGCs, however, neurite outgrowth has not been stimulated significantly [3-4]. It is paramount to design a platform for the sustained delivery of neurotrophins [5]. The aim of this study was to fabricate bioactive surfaces, which would stimulate neurite outgrowth, by using heparin to bind to amine groups. Then, NGF or BDNF were bound electrostatically, or immobilized, to heparin. To characterize the bioactive surface, water contact angle (WCA), XPS analysis, and ELISA assay were performed. To study the effect of the bioactive surface, chick embryo dorsal root ganglia (DRGs) were seeded on bioactive surfaces, and neurite outgrowth was measured [6]. The bioactive surface was successfully fabricated, confirmed by WCA and XPS analysis. ELISA assay revealed that surfaces with immobilized NGF 1 ng/mL showed no neurotrophin release until 168 h, where 1% of NGF was detected. DRGs cultured on immobilized NGF 1 ng/mL developed neurites significantly longer in comparison to control and other test surfaces, possibly due to a low initial burst release, and for the sustained activation of a non-internalized NGF-TrkA complex [6]. In conclusion, locally delivered NGF and BDNF, at relatively low concentrations, is a promising and scalable approach to support sufficient neurite outgrowth for modifying medical implantable devices for nerve regeneration.

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Abstract **#199**

TOPOGRAPHICALLY CONTROLLED NANOFIBROUS MEMBRANES TO MIMIC THE EPIDERMAL STEM CELL MICROENVIROMENT OF THE RETE RIDGES

David Hiram Ramos$_P$[1], Sheila MacNeil[1], Frederik Claeyssens[1], Ilida Ortega Asencio[1]

[1] *The University of Sheffield*

Corresponding author's email: dhramosrodriguez1@sheffield.ac.uk

Adult stem cells reside on specific microenvironments at the interface between the epidermal and dermal layers of the skin called rete ridges. These microenvironments control stem cell quiescence via cell-cell interactions and soluble factors [1]. Although current approaches replicate one or more components of the rete ridges, the design of a biomedical device that mimics the micro and nanostructure of the stem cell microenvironment is yet to be explored in vitro [2] [3]. The aim of this project is to study the regenerative capabilities of topographically controlled electrospun scaffolds (TCESs) using an in vitro tissue-engineered skin model.

Polycaprolactone TCES were electrospun using poly(methyl methacrylate) patterned collectors. Skin models were prepared using human dermal fibroblasts and keratinocytes onto de-epidermised acellular human dermis. Additionally, control models without the scaffold were burned at day 7 followed by implantation of the TCES. H&E histology and ICC were used to evaluate tissue structure.

Histology sections showed cell infiltration from the TCES towards the dermal component. The microfeatures formed, although wider, showed similar depths. Topographical cues proved to be a key factor for tissue integration, with low cell attachment and no infiltration for conventional plain scaffolds. When burned at day 7, control skin models were not able to repopulate the damaged areas, whereas TCES controls showed improvements in the regeneration of the epidermal component and formation of basic microenvironments. This work demonstrates that the inclusion of topographical cues within scaffold design is a promising approach to recreate to a degree the morphology of the Rete Ridges.

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Abstract **#201**

DEVELOPMENT OF DECELLULARIZED WHOLE HUMAN LIVERS FOR INCREASING DONOR POOL

Ayli Acun[P][1,2,3], Ruben Oganesyan[1,2,3], Basak E. Uygun[1,2,3]

[1] *Center for Engineering in Medicine and Surgery*

[2] *Massachusetts General Hospital*

[3] *Harvard Medical School*

Corresponding author's email: aacun@mgh.harvard.edu

Eight patients die daily awaiting liver transplant. Although there is donor liver scarcity, thousands of livers are discarded due to conditions such as advanced age. To ameliorate donor scarcity, we aim repurposing discarded human donor livers through whole liver decellularization and subsequent recellularization with patient-specific cells. The purpose of this study is to assess the effect of advanced donor age on ECM composition and structural organization as the variation in quality and age will affect translating this approach to clinical settings. To achieve this, we have decellularized 3 young (<44 years old) and 6 aged (>44 years old) whole human livers through a 4 day-long protocol of perfusion with sodium dodecyl sulfate through portal vein and hepatic artery. Total DNA, collagen, and glycosaminoglycan contents were assessed, and mass spectrometry and histology were used to examine age-related changes in structural composition and organization of the liver matrix. Our results showed that more than 90% of DNA removal from all livers. Aged livers had a less homogenous decellularization and higher collagen and glycosaminoglycan content. Histology analysis showed that the structural integrity was preserved in livers regardless of age while aged livers had higher remaining fat content and a denser collagen network. Overall, our results indicate that there are age-related changes in liver ECM composition and structure. Through further characterization of livers from different aged donors, the widely varying discarded donor livers can be classified, thus be used effectively as decellularized scaffolds to develop human liver substitutes with high functionality and regenerative capacity.

Abstract **#204**

EFFECTS OF DYNAMIC ENVIRONMENT AND ELECTROMAGNETIC FIELD EXPOSURE ON FABRICATION OF FUNCTIONAL SUBSTITUTES BASED ON BONE-DERIVED SCAFFOLD

Kedong Song[P][1], Xueyan Hu[1], Shuangjia Yang[1], Jie Xu[1], Xiangqin Li[1], Tianqing Liu[1]

[1] State Key Laboratory of Fine Chemicals, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian 116024, China;

Corresponding author's email: kedongsong@dlut.edu.cn

Biological treatment using engineered osteochondral composites with the dynamic microenvironment has received growing attention. In this study, a bone-derived scaffold with good biocompatibility and osteoinductive capability was constructed. Porcine femur in distal metaphysis was treated by chemical decellularization, deproteinization, decalcification, and degreasing to obtain the bone-derived scaffold. It was found that bone-derived scaffold was eluted and had good biocompatibility which can serve as a suitable material to support bone tissue. We also designed a spinner bioreactor for the in vitro modeling of the microenvironment which can promote adipose mesenchymal stem cell (ADSCs) recruitment from the simulated bone marrow compartment into the bone-derived scaffold under physiological flow conditions. Results demonstrated that at the end of 14 days in dynamic microenvironment culture, the ADSCs had achieved 1.73-fold expansions than in the static culture. And the ADSCs-bone derived scaffold composites had better proliferation and differentiation under dynamic microenvironment via spinner flask than static culture, and were able to differentiate into osteoblasts better with extensive mineralized nodules forming the bone aggregates over 3 weeks postosteogenic induction. The electromagnetic field (EMF) stimulation of ADSCs was evaluated in vitro. The results showed that 75Hz/1mT EMF fields stimulated ADSCs could enhance osteogenic proliferation and differentiation. Results also shown that the numbers of ADSCs expanded under 1mT EMFculture were 1.55 times in DMEM and 1.64 times in OM more than EMF-free culture. In conclusion, the designed EMF-stimulated dynamic microenvironment can served as an suitable investigational tool for the screening of other biomaterial-based tissue engineering strategies.

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Abstract **#205**

EVALUATION OF INHIBITORY EFFECTS OF GENIPOSIDE ON A TUMOR MODEL OF HUMAN BREAST CANCER BASED ON 3D PRINTED CS/GEL HYBRID SCAFFOLD

Tianqing Liu[P][1], Kena Lv[1], Jingjing Zhu[1], Xiangqin Li[1], Kedong Song[1]

[1] *1 State Key Laboratory of Fine Chemicals, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian 116024, China;*

Corresponding author's email: kedongsong@dlut.edu.cn

Traditional Chinese medicine therapy, which can serve as adjuvant therapy for cancer treatment, has no obvious side effects on the human body. Geniposide (GEN), one of the main iridoid glycosides in gardenia fruit, has been widely reported to have anti-cancer effects. In this study, we aimed to inspect whether GEN could inhibit proliferation and promote the apoptosis of human breast cancer cells (MCF-7). In order to better predict the efficacy of GEN, we have prepared the Cs/Gel composite scaffolds by 3D printing technology to mimic the MCF-7 cell growth microenvironment. The prepared Cs/Gel scaffold has good mechanical properties and biocompatibility, which can provide a more accurate platform for drug screening. The semi-inhibitory concentration (IC50) evaluated by CCK-8 assay was 16.06 mg/mL (24 h), 14.85 mg/mL (48 h), and 13.14 mg/mL (72 h). After exposed to GEN for 48h, the cancer cell survival rate reduced from 69.15 ± 2.86% (13 mg/mL) to 20.97 ± 3.24% (16 mg/mL). Although the inhibitory effect was weaker in the 3D culture system, it also managed to inhibit cell proliferation and induce cell apoptosis. Besides, Live/Dead staining, Hematoxylin-Eosin (H&E) staining and SEM evaluation were also conducted to estimate the anti-cancer effect of GEN in 2D and 3D cultures. The results indicate that GEN has an anti-cancer effect based on a time- and dose-dependent manner.

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Abstract **#206**

TRIPLY PERIODIC MINIMAL SURFACE (TPMS) BASED STAINLESS-STEEL SCAFFOLDS FOR BONE TISSUE IMPLANT APPLICATIONS

Sanjairaj Vijayavenkataraman[1,2]

[1] *Division of Engineering, New York University Abu Dhabi, Abu Dhabi, UAE*

[2] *Department of Mechanical and Aerospace Engineering, Tandon School of Engineering, New York University, Brooklyn, USA*

Corresponding author's email: vs89@nyu.edu

Triply Periodic Minimal Surfaces (TPMS) based porous scaffolds are expected to provide better strength, stiffness, and microarchitecture for cell proliferation to repair bone fractures [1-2]. Here, we describe an effective method to generate and test the biocompatibility of EOS stainless steel 316 based TPMS scaffolds using additive manufacturing technology. Five different types of porous TPMS scaffolds: cubic (control), gyrosheets, IWP, diamond, and gyrosolids were designed, 3D printed, and tested for its structure, mechanical properties and biocompatibility. Though the designed porosity of the scaffolds was 50%, the actual porosity of the 3D printed scaffolds showed slight variations ranging from 4 to15%. SEM image analysis of the scaffold surface pore sizes showed a range of 150 – 800 µm, well suited to facilitate the cell proliferation and vascularization. In compression tests, all five types of scaffolds showed Young's modulus similar to native bone tissue, ranging from 2.3 - 4.8GPa, while the toughness of the scaffolds was in the rage of 40-120 MJ/M3, suitable to resist bone fractures. The plateau stress assessment of the TPMS scaffolds revealed a stiffness ranging from 95 - 112.59 MPa, which denotes superior load-bearing ability of these structures. The cell toxicity (live-dead count) and the cell proliferation assays (MTT assay) using MEF cell culture in the scaffolds showed that the scaffolds were non-toxic, biocompatible and favor cell growth. With a porous structure, reduced weight, and enhanced cell proliferation, our results indicate that the 3D printed SS-based TPMS scaffolds have high potential to be used as permanent bone implants.

Keywords

TPMS; 3D Printing; Bone implants

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Abstract **#207**

POTENTIAL OF BIOINSPIRED POLYPHENOLIC SURFACE MODIFICATIONS FOR ANTI-INFLAMMATORY AND ANTIMICROBIAL TI DENTAL IMPLANTS

Florian Weber[P][1], Alejandro Barrantes[2], Hanna Tiainen[1]

[1] *Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, Norway*

[2] *Clinical Oral Research Laboratory, Institute of Clinical Dentistry, University of Oslo, Norway*

Corresponding author's email: florian.weber@odont.uio.no

Biofilm-film associated infections around dental implants lead to chronic inflammatory response that results in significant tissue destruction and reduced wound healing capacity even after the biofilm has been removed.1 In the quest for the future generation of implant surfaces, continuous efforts are made studying plant polyphenolic molecules due to their combined antioxidant and antimicrobial properties. A variety of these molecules have been shown to mediate inflammatory conditions by scavenging reactive radical species and reducing the expression of pro-inflammatory cytokines.2, 3 Further, their interaction with microbial cell walls, and inhibition of essential enzymes and signaling pathways results in antimicrobial properties.4

While these effects have been mainly studied in vitro using dissolved polyphenolic molecules, our aim is to functionalize implant surfaces with these molecules. Therefore, we investigated the oxidative polymerization of pyrogallol and created novel silica-phenolic networks using tannic acid. The use of silica-TA networks allowed us to lower the reaction pH and thereby avoid oxidation and polymerization processes, which led to an increased deposition efficacy. As a result of the reduced pH, the antioxidant capacity of TA coating was increased.

Utilizing this continuous, surface independent deposition process of TA and PG, a local reservoir of active molecules can be built. The subsequent release of active molecules was shown to be more sensitive to acidic environment for silica-TA networks compared to PG coatings. Since antimicrobial and anti-inflammatory properties of polyphenolic coatings have been related to released molecules,5 increased layer thicknesses have a higher potential to reduce bacterial colonization and relieve inflammatory conditions.

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Abstract **#208**

OPTIMAL CULTURE CONDITIONS OF COLON CANCER CELL LINE TO INCREASE THE CANCER STEM CELL POPULATION IN VITRO

Ping - Tse Hsieh$_P$[1], Jia - Hua Wang[1], Ting - Yeh Chen[1], Akon Higuchi[1,2,3]

[1] *Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli, Taoyuan 32001, Taiwan*

[2] *Center for Emergent Matter Science, RIKEN, Wako, Saitama 351-0198, Japan*

[3] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*

Corresponding author's email: a5898321@gmail.com

Tumors contain a small population of cancer stem cells (CSCs), which express stem cell properties, and possess a self-renewing capacity and differentiation abilities. CSCs that exist as a different population to cancer cells are responsible for tumor generation and also result in relapse and metastasis that gives rise to new tumors. However, CSCs only comprise 1%–5% of the total tumor cell population. Therefore, finding the optimal environment for CSCs to proliferate is extremely important. We investigated patient-specific cancer cell line to target the CSCs for future application of chemotherapy drug selection for each specific colon cancer patient.

We evaluated the effect of cancer stem cells through culturing with different extracellular matrix (ECM) and different seeding density. Different ECM have specific physical and biochemical properties that provide structural support of cells and play important roles in cell survival, migration, and differentiation. Single cancer cell can secrete paracrine by itself, which facilitates the growth; hence, we want to observe if the ECM-coated dish could enhance the cancer cell proliferation. We analyzed the cell attachment ratio on different ECM-coated surface and found Matrigel was the best ECM for establishment and proliferation of cancer cell colonies. Furthermore, we discovered the seeding density, 100 cells/well, has the greatest expansion fold and shorter doubling time on each ECM-coated surface. Consequently, we continue operating flow cytometry (CD44, CD133) to verify CSC expression and ultimately find the optimal environment for CSC proliferation and establishment.

Keywords

Cancer; Extracellular matrix

Abstract **#209**

THREE-DIMENSIONAL PRINTING OF LITHIUM-DOPED CALCIUM SILICATE POROUS SCAFFOLD FOR OSTEOCHONDRAL REGENERATION

Yun-Ting Chou[P][1,2], Yeng-Hong Lin[2,3], Tsung-Li Lin[4,5], Ming-You Shie[2,6]

[1] *Institute of Dental and Oral Health, China Medical University*

[2] *XD Center for Medical Research and Translation, China Medical University Hospital*

[3] *The Ph.D. Program for Medical Engineering and Rehabilitation Science, China Medical University*

[4] *Department of Orthopedics, China Medical University Hospital*

[5] *Department of Sports Medicine, China Medical University*

[6] *School of Dentistry, China Medical University*

Corresponding author's email: tingsmile666@gmail.com

Cartilage wear is a common joint disease, which may lead to osteoarthritis, and may even damage the subchondral bone as it progresses. Research related to bioceramics have its own values in tissue engineering, especially, calcium silicate-based materials are attractive due to their unique osteoinductivity. Three-dimensional (3D) printing technology has the advantages of fast, accurate and controllable manufacturing process, and has been widely used in cartilage tissue engineering. In this study, we fabricate the lithum-doped calcium silicate porous scaffold by 3D printing technology for the repairing of subchondral defects. The experimental results found that the scaffold have a mechanical structure with sufficient strength, as well as accelerated proliferation and differentiation of Wharton's jelly mesenchymal stromal cells. The animal model with subchondral defects also shown that the lithium-doped calcium silicate scaffold has the better ability to enhance new osteochondral regeneration in vivo. In summary, litium-doped bioceramic porous scaffolds could be a promising biomaterial for the osteochondral regeneration.

Keywords

3D printing; calcium silicate; magnesium

Abstract **#210**

A VERSATILE MODEL OF CARDIAC TISSUE FROM HIPSC-DERIVED CARDIOMYOCYTES WITHIN PERICARDIAL MATRIX HYDROGELS DOPED WITH HYDRAZIDE FUNCTIONALIZED MWCNTS FOR PHARMACEUTICAL SCREENING AND TISSUE ENGINEERING APPLICATIONS

Kaveh Roshanbinfar$_P$[1], Oommen Podiyan Oommen[2], Jöns Hilborn[3], Felix B Engel[1]

[1] *Experimental renal and cardiovascular research group, Translational Research Center, Department of Nephropathology, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany*

[2] *Tampere University of Technology, Tampere, Finland*

[3] *Department of Polymer Chemistry, Uppsala University, Uppsala, Sweden*

Corresponding author's email: k.roshanbinfar@gmail.com

Cardiovascular diseases represent a significant socio-economic burden. For instance, following losing cardiac cells after an infarct, substantial work has been invested in optimizing and advancing cell transplantation strategies to restore heart function. A particular concern is that the implantation of cells using a non-electroconductive matrix potentially induces arrhythmia. Here, we generated a conductive biohybrid hydrogel based on hydrazide-functionalized multiwall carbon nanotubes (MWCNTs) incorporated into decellularized and digested pericardial matrix. Furthermore, we demonstrate that this platform, either in 2D or 3D, is a suitable environment for maturing human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes. hiPSC-derived cardiomyocytes showed an enhanced contraction amplitude (>500%) on conductive hydrogels compared to cells cultured on Matrigel®, accompanied by enhanced cellular alignment, elevated connexin 43 expressions, and improved sarcomere organization suggesting maturation of the hiPSC-derived cardiomyocytes. Sarcomeric length of these cells increased from 1.3 to 1.7 µm. Furthermore, we generated 3D cell-laden tissues that positively responded to external electrical stimulation and exhibited improved calcium handling compared to tissues without MWCNTs. We then utilized these 3D models of cardiac tissues to study the effect of four different candidate drugs on beating properties. Our engineered tissues showed a more apparent detectable response to drugs that either promote (isoproterenol and epinephrine) or inhibit (verapamil and nifedipine) heart beating rate compared to tissues without carbon nanotubes. Our data collectively indicate that our biohybrid hydrogels consisting of solubilized pericardial matrix and electroconductive positively charged hydrazide-conjugated MWCNTs provide a promising material for generating 3D models of cardiac tissue and stem cell-based cardiac tissue engineering.

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Abstract **#211**

PHOTO-CURABLE 3D PRINTED Γ-POLYGLUTAMIC ACID/CALCIUM SILICATE SCAFFOLDS FOR PROMOTING BONE REGENERATION

Hsin-Yi Huang[P][1,2], Cheng-Yu Chen[2], Chun-Pin Lin[3], Ming-You Shie[2,4]

[1] *Institute of Dental and Oral Health, China Medical University, Taiwan*

[2] *XD Center for Medical Research and Translation, China Medical University Hospital, Taiwan*

[3] *Department of Dentistry, National Taiwan University*

[4] *School of Dentistry, China Medical University, Taiwan*

Corresponding author's email: tracyfamly@gmail.com

A hydrogel scaffold with good mechanical properties, biocompatibility and biodegradability can effectively promote tissue regeneration is essential for hard tissue repair and regeneration. However, there is no bone scaffold that can integrate all these characteristics. Therefore, we hope to use γ-PGA and calcium silicate to produce a hydrogel with adequate strain, osteoinductivity and osseointegration. γ-PGA hydrogel has excellent water retention capacity, biocompatibility and biodegradability. We improve the mechanical properties by adding calcium silicate. The good wrapping and drug loading characteristics of γ-PGA for encapsulating the cells and calcium silicate to form a repair microenvironment inside. Herein, our study used 3D printing to fabricate scaffolds made up of γ-PGA/CS. It was reported that the scaffold was not only able to enhance bone cell proliferation but also induce differentiation by the silicon ions released from calcium silicate. From the results of implantation in animal model showed that the scaffold could promote blood vessel and bone regeneration. Based on the above, it is known that the γ-PGA hydrogel scaffold could be serve as a potential and biological material for bone tissue regeneration applications.

Keywords

Hydroxy apatite; Calcium sulfate; 3D printing

Abstract **#212**

DEVELOPMENT OF SCHWANN CELL-CONTAINED CONDUCTIVE BIO-INK FOR PERIPHERAL NERVE REGENERATION

Chao-Kai Liao[P][1,2], Chie-Chang Chen[2], Yueh-Sheng Chen[3], Ming-You Shie[2,4]

[1] *Master Program for Biomedical Engineering, China Medical University*

[2] *XD Center for Medical Research and Translation, China Medical University Hospital*

[3] *Department of Cosmeceutics, China Medical University*

[4] *School of Dentistry, China Medical University*

Corresponding author's email: dvd10a21@gmail.com

Peripheral nerve injury is one of the problems to be solved in neuro medicine. The current gold standard in clinical practice is autologous transplantation, but the problems of insufficient supply sites and mismatched sizes have made nerve conduits widely used in clinical practice. The rapid improvement of 3D printing technology in recent years has also made nerve conduits an alternative process. Among conductive polymers, poly(3,4-ethylenedioxythiophene): poly(styrenesulfonate) (PEDOT: PSS) has considerable stability and high conductivity, and is a promising conductive material in biomedical research. In this study, conductive nerve conduit with Schwann cells was prepared through 3D bioprinting technology. During electrical stimulation, Schwann cells in the nerve conduit were stimulated to cell proliferate, and expected to secrete more nerve growth factor (NGF). Besides, nerve regeneration was evaluated via the rat animal model of peripheral nerve injury. The results showed that the conductive nerve conduit with Schwann cells had good biological activity, and the Schwann cells also had good performance in immunofluorescence staining results after electrical stimulation. In the in vivo study, conductive nerve conduits with Schwann cells performed better in electrical stimulation. We hope the developed Schwann cell-contained conduit has good potential in nerve tissue engineering in the future.

Keywords

Schwann cells; Cell block; 3D printing

Abstract **#214**

FUNCTIONAL FIBRIN-BASED HYDROGELS FOR CONTROLLING CELL/BIOMATERIAL INTERACTIONS IN BIOHYBRID IMPLANTS

Svenja Weinp[1,2], Pia Hefer[1,2], Hanna Malyaran[1,2], Norina Labude-Weber[1,2], Stephan Rütten[3], Nicole Terefenko[4,5], Miriam Al Enezy-Ulbrich[4,5], Andrij Pich[4,5], Sabine Neuss[1,2]

[1] Helmholtz Institute for Biomedical Engineering, BioInterface Group, RWTH Aachen University,Pauwelsstrasse 20, 52074 Aachen, Germany

[2] Institute of Pathology, RWTH Aachen University, Pauwelsstrasse 30, 52074 Aachen, Germany

[3] Electron Microscopic Facility, University Clinics, RWTH Aachen University, Pauwelsstrasse 30, 52074 Aachen, Germany

[4] DWI - Leibniz Institute for Interactive Materials, RWTH Aachen University, Forckenbeckstraße 50, 52074 Aachen

[5] Institute for Technical and Macromolecular Chemistry, Research Area Functional and Interactive Polymers, RWTH Aachen University, Forckenbeckstraße 50, 52074 Aachen

Corresponding author's email: svwein@ukaachen.de

Today's implant research faces the challenge of improving both the biocompatibility and reproducibility of implants. Biohybrid implants consisting of a technical and a patient-specific component are suitable for this purpose. The aim is the development of a biohybrid heart valve based on a hydrogel matrix with textile reinforcement. A functional toolbox for fibrin-based hydrogels is developed and the direct cell/biomaterial interaction and the long-term behaviour of the cells in the in vitro implant is investigated.

More concrete, new biohybrid hydrogels from fibrinogen and poly(N-vinylpyrrolidone) copolymers are used. For later application of the heart valve, smooth muscle cells (SMC) are required. Therefore, human stem cells are cultured inside the hydrogels for myogenic differentiation induced by the specific growth factors TGF-β1 and BMP4 over 21 days. We focus on the distinction between the contractile and the synthetic phenotype of SMC as the contractile type is mandatory for functional heart valves. Vascularization is essential for the supply of the construct after implantation, thus, the formation of capillary-like structures by endothelial cells in contact with feeder layers of stem cells within the gel is analyzed.

First experiments show long-term stable hydrogels, which trigger the successful myogenic differentiation of mesenchymal stem cells.

The analysis is performed by RT-qPCR, immunofluorescence and scanning electron microscopy as well as two-photon microscopy, with emphasis on the expression of the contractile and synthetic markers.

Compared to conventional heart valves, biohybrid heart valves offer the decisive advantage of personalization using the patient's own stem cells and thus represent a future-oriented technology.

Keywords

biohybrid heart valve; fibrin gel; angiogenesis

Abstract **#215**

ENHANCEMENT OF BIOFUNCTIONALITY TO BONE TISSUE REGENERATION BY 3D PRINTING MULTI-CALCIUM SILICATE SCAFFOLDING ARCHITECTURE

Yen-Hong Lin[P][1,2], Yi-Wen Chen[1,3], Ming-You Shie[1,4]

[1] *XD Center for Medical Research and Translation, China Medical University Hospital, Taiwan*

[2] *The Ph.D. Program for Medical Engineering and Rehabilitation Science, China Medical University, Taiwan*

[3] *Graduate Institute of Biomedical Sciences, China Medical University, Taiwan*

[4] *School of Dentistry, China Medical University, Taiwan*

Corresponding author's email: roger.lin0204@gmail.com

Calcium silicate (CS) has attracted researchers' attention for its favorite bone adaptability and bone formation. Our team has previously developed the magnesium-doped CS (MgCS), which can stimulate the osteogenic differentiation and angiogenesis of human periodontal ligament cells by the released Mg ions, but is concerned about their mechanical strength and rapid biodegradability. On the other hand, we had also created strontium-doped CS (SrCS), which has more high mechanical properties compared with pristine CS, and better biological functions for osteogenic differentiation. Therefore, the methodology here was constructed multi-CS in a single scaffold (MSCS) using direct-ink-writing method. The printed outer struts of the scaffold consist of MgCS, while the central structure consists of SrCS to prevent excessive degradation and collapse of the scaffold, thereby affecting new bone tissue ingrowth. The results confirmed that the strain-stress curve was much higher and the degradation time was prolonged compared with the MgCS scaffold. The doped ions (Sr, Mg) released from MSCS have a huge impact on the behavior of Wharton's jelly mesenchymal stem cells (WJMSC). The MSCS also significantly promoted cell growth, and alkaline phosphatase activity and more calcium deposits were found on the surface of the scaffold. Moreover, the establishment on rabbit critical-size bone defect model demonstrated that more new bone tissue ingrowth by the analysis of BV/TV and histological staining results. This study showed that the 3D printed multi-CS scaffolds could modulate on degradability and maintain the strength of the entire structure, while ions synergistically promote the osteoinduction in vitro and in vivo.

Keywords

calcium silicate; magnesium; strontium

Abstract **#216**

SUPRAMOLECULAR AND PHOTO-SENSITIVE HYDROGELS BASED ON A CUSTOM-MADE POLY(ETHER URETHANE) AND A-CYCLODEXTRINS FOR THE SUSTAINED RELEASE OF CURCUMIN

Gianluca Ciardelli[P][1], Alessandro Torchio[1], Claudio Cassino[2], Mario Lavella[3], Monica Boffito[1]

[1] *Politecnico di Torino*

[2] *Università del Piemonte Orientale*

[3] *Università degli Studi di Bergamo*

Corresponding author's email: gianluca.ciardelli@polito.it

The design of supramolecular hydrogels represents a valuable strategy to develop therapeutic platforms characterized by remarkable processability and high drug encapsulation capacity. The implementation of α-cyclodextrins (CDs) and poly(ethylene oxide) (PEO)-based polymers to form highly-hierarchical hydrogels composed of poly(pseudo)rotaxanes has been exploited to this aim1-3. Nonetheless, the reversible nature of these networks could hinder their stability in biological environments. In this work, a supramolecular network based on a custom-made photo-curable poly(ether urethane) (PEU) and CDs was designed with the aim to develop hydrogels with highly tuneable physical properties and release kinetics. Poloxamer 407(amphiphilic triblock co-polymer composed of PEO (70%) and poly(propylene oxide)), 1,6-diisocyanatohexane and 2-hydroxyethyl methacrylate as end-capping molecule were utilized for PEU synthesis. The formation of poly(pseudo)rotaxanes based on PEU and CDs was demonstrated through X-Ray Diffraction crystallography, Infrared and Proton Nuclear Magnetic Resonance spectroscopies. Supramolecular hydrogels were formulated with CDs ranging between 7 and 10% w/v at low PEU content (1-5% w/v), showing fast gelation kinetics (ranging from minutes to few hours). CD concentration of 8% w/v was selected as the best compromise between good mechanical and self-healing properties. Photo-rheological characterizations (365 nm, 10 mW/cm2) were conducted, showing good photo-responsiveness. Successful encapsulation and protection from degradation of curcumin (up to 570 µg/ml) were attained exploiting its complexation with CDs and PEU4,5. Tuneable release profiles of curcumin (up to 23 days) were obtained through photo-crosslinking, thus making such platforms promising for cancer treatment6,7. Finally, the formulation containing PEU at 5% w/v resulted to be suitable for extrusion-based 3D-printing.

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Abstract **#217**

DEVELOPMENT OF HYALURONIC ACID/WATERBORNE POLYURETHANE 3D PRINTED HYBRID SCAFFOLDS FOR CARTILAGE REGENERATION

Yu-Hao Lai[P][1,2], Yu-Fang Shen[3], Gilin Lin[1], Ming-You Shie[4,2]

[1] *Department of Chemistry, National Chung Hsing University, Taichung, Taiwan*

[2] *XD Center for Medical Research and Translation, China Medical University Hospital, Taichung, Taiwan*

[3] *Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan*

[4] *School of Dentistry, China Medical University, Taichung, Taiwan*

Corresponding author's email: joeyman772000@gmail.com

Cartilage is highly specific, but has limited supply of nerves, blood vessels, lymphatics, and low metabolic activity. This avascular structure causes a lack of precursors and vegetative cells, thus the ability of cartilage self-regeneration is severely limited. Due to the lack of nerves and blood vessels, When the pain felt significantly, the extent of damage has usually reached to the subchondral bone, so minor cartilage defects are generally difficult to detect. Despite of using drug or surgical treatment can not restore the function and structure of cartilage in current. With the development of tissue engineering, the possibility of cartilage tissue regeneration has been improved. In order to clinical application, it is necessary to develop implant scaffold which having good biocompatible, biodegradable, and high mechanical properties. In this case, we prove that the photosensitive liquid resin of a hyaluronic acid-based polyurethane for the feasibility of cartilage regeneration. The ability of chondrogenesis with human chondrocyte-laden hybrid scaffold was evaluated in vitro and showed well-proliferation at day 7. Up to 8 weeks, degradation comply with the degradation rate of cartilage regeneration. Also, strength of the hybrid scaffold in the compression test achieved 25 Mpa. Moreover, we discovered the bone recovery surround by cartilage in vivo experiments against defected articular cartilage rabbit model. In this article, the fabricated hyaluronic acid-based polyurethane using DLP printer is a prospective way that could be applied in tissue enginerring and development of cartilage regeneration.

Keywords

Digital Light Processing; Waterborne polyurethane; Hyaluronic acid

Abstract **#218**

AN SDF-1A GENE-ACTIVATED COLLAGEN SCAFFOLD RESTORES PRO-ANGIOGENIC WOUND HEALING FEATURES IN HUMAN DIABETIC ADIPOSE-DERIVED STEM CELLS

Michael B. Keogh[P][1], Ashang Laiva[1], Fergal J. O' Brien[2]

[1] *RCSI Bahrain*

[2] *RCSI*

Corresponding author's email: mkeogh@rcsi.com

Diabetic foot ulcer is one of the leading causes of leg amputation and mortality in diabetic patients. Autologous stem cell therapy holds some potential to be a solution to this problem, however diabetic stem cells are relatively dysfunctional and restrictive in their wound healing abilities. This study explores the wound healing abilities of a novel pro angiogenic gene activated scaffold (GAS) previously developed in our lab [1] to stimulate human diabetic adipose stem cells (ADSCs) towards wound healing.

Our GAS consisted of a collagen composite containing the pro-angiogenic stromal-derived factor-1 alpha. This was seeded with diabetic ADSCs and compared to healthy ADSC on the gene-free scaffold over 2 weeks period. We analysed functional responses including SDF-1α mediated signaling, bioactive factors production, proangiogenic bioactivity of secreted factors, matrix deposition and proteome profiling,

We found that SDF-1GAS could restore proangiogenic regenerative response in the human diabetic ADSCs, similar to the healthy ADSC gene-free scaffolds. SDF-1α gene-activated scaffold induced the overexpression of SDF-1α in diabetic ADSCs and engaged CXCR7, causing downstream signaling of β-arrestin as effectively as the transfected healthy ADSCs. The transfected diabetic ADSCs also effectively stimulated angiogenesis in endothelial
cells while undergoing matrix remodeling (reduction in fibronectin and increase collagen IV).

Conclusion: We show that the SDF-1α gene-activated scaffold can overcome the deficiencies associated with diabetic ADSCs paving the way for autologous patient stem cell therapies in combination with novel biomaterials to treat diabetic foot ulcers.

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Abstract **#219**

CONSTRUCTION OF 3D FIBROUS PCL SCAFFOLDS BY COAXIAL ELECTROSPINNING FOR PROTEIN DELIVERY

Mehrnoosh Rafiei$_P$[1]

[1] *Department of Chemical and Petroleum Engineering, Sharif University of Technology*

Corresponding author's email: mehrnooshrafiei@gmail.com

In this study, a three-dimensional tablet-like porous scaffold, comprising core-shell fibers to host proteins inside the core, was developed. The fabrication method involved the novel combination of coaxial and wet electrospinning in a single setting. Poly (ε-caprolactone) was chosen as the based polymer and bovine serum albumin was used as a model protein. These 3D tablet-like scaffolds exhibited adequate porosity and suitable pore size for cell culture and cell infiltration, in addition to appropriate mechanical properties for cartilage tissue engineering. The effects of different parameters on the behavior of the system have been studied and the 3D scaffold based on the core-shell fiber was compared with that based on the matrix fiber. The core-shell structure showed superior performance in comparison to the matrix structure by sustaining protein release kinetics at least for 12 days in PBS. The results from in vitro cell cytotoxicity study revealed that the presented scaffold was biocompatible and non-toxic. Coaxial electrospinning was shown to be a versatile technique in achieving the delivery of biochemical signals in a controlled manner for the regeneration of cartilage. These 3D tablet-like PCL scaffolds incorporated with protein solutions are engineered systems that closely mimic the characteristics of cartilage tissue.

Keywords

Wet electrospinning; 3D porous PCL scaffold; BSA sustained release

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Abstract **#220**

SDF-1A GENE-ACTIVATED COLLAGEN SCAFFOLD DRIVES FUNCTIONAL DIFFERENTIATION OF HUMAN SCHWANN CELLS FOR WOUND HEALING APPLICATIONS

Laiva Ashang Luwang[P][1], Fergal O'Brien[2,3], Michael Keogh[1]

[1] *Royal College of Surgeons in Ireland-Bahrain*

[2] *Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin*

[3] *Trinity Centre for Bioengineering, Trinity College Dublin, Ireland*

Corresponding author's email: lluwang@rcsi-mub.com

Enhancing angiogenesis is the prime target of current biomaterial-based wound healing strategies (1). However, these approaches largely overlook the angiogenic role of the cells of the nervous system. Therefore, we explored the role of a collagen-chondroitin sulfate scaffold functionalized with a pro-angiogenic gene stromal-derived factor-1 alpha (SDF-1α) – an SDF-1α gene-activated scaffold on the functional regulation of human Schwann cells (SCs). A preliminary 2D study was conducted by delivering plasmids encoding for the SDF-1α gene into a monolayer of SCs using polyethyleneimine-based nanoparticles. The delivery of the SDF-1α gene into the SCs enhanced the production of pro-angiogenic VEGF. Subsequently, we investigated the impact of SDF-1α gene-activated scaffold (3D) on the SCs for two weeks, using a gene-free scaffold as control. The transfection of the SCs within the gene-activated scaffold resulted in transient overexpression of SDF-1α transcripts and triggered the production of bioactive VEGF that enhanced endothelial angiogenesis. The overexpression of SDF-1α also caused a transient activation of the transcription factor c-Jun and supported the differentiation of SCs towards a repair phenotype. This was characterized by elevated expression of neurotrophin receptor p75NGFR. During this developmental stage, the SCs also performed an extensive remodelling of the basement matrix (fibronectin, collagen IV, and laminin) to enrich their environment with the pro-neurogenic matrix protein laminin, revealing an enhanced pro-neurogenic behaviour. Together, this study shows that SDF-1α gene-activated scaffold is a highly bioinstructive scaffold capable of enhancing pro-angiogenic regenerative response in human SCs for improved wound healing.

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Abstract **#221**

3D PRINTED PCL/TCP AND PCL/GELMA COMPOSITE SCAFFOLDS FOR CRANIOFACIAL BONE RECONSTRUCTION

Dongxu Ke[P][1], Xingzhi Liu[2], Peixin Liu[2], Wenguo Cui[3], Xi Yang[1]

[1] *Novaprint Therapeutics*

[2] *Soochow University*

[3] *Shanghai Ruijin Orthopedic Institute*

Corresponding author's email: jason.ke@3dnovaprint.com

Craniofacial bone reconstruction is a clinical challenge due to the demand for fast bone induction, precise shape fidelity, and patients' specific customization. Extrusion-based 3D printing is a promising technique to create patients' specific implants for craniofacial bone reconstruction, however, the current bone tissue engineering scaffolds produced by this method lacks osteoinductivity and hydrophilicity that are crucial for bone reconstruction. In this study, different percentage of TCP was added to PCL for producing PCL/TCP composites. Then macroporous PCL and PCL/TCP composite scaffolds were prepared by an extrusion-based 3D printer. The printed PCL scaffolds were fixed in a customized mold with gelatin methacryloyl (GelMA) followed with UV crosslinking at 365 nm in order to achieve PCL/GelMA composite scaffolds. Physical and chemical characterizations, including gel permeation chromatography (GPC), compressive strength, contact angle, and X-ray diffraction (XRD), of these scaffolds were evaluated. Besides, PCL, PCL/TCP, and PCL/GelMA scaffolds were implanted in a critical-sized calvarial defect model of rats for up to eight months. In vivo characterizations contained degradation kinetics, micro-CT, histology, and immunochemistry. Results indicated that PCL/TCP and PCL/GelMA composite scaffolds showed comparable compressive strength (3-5 MPa) to human cancellous bone. The addition of GelMA filled the macroporosity (~500 um) of PCL scaffolds with microporosity (50-100 um), which enhanced the hydrophilicity of scaffolds. Meanwhile, PCL/TCP and PCL/GelMA composite scaffolds exhibited better craniofacial bone reconstruction after implantation in rats' calvarial defect model for eight months through histological and immunochemical analysis of H&E, Goldern's trichrome, collagen I, vWF stainings.

Abstract **#222**

3D-PRINTING OF SHAPE MEMORY POLYMERS AS A TOOL TOWARDS MINIMALLY INVASIVE BREAST RECONSTRUCTION

Coralie Greant$_P$[1], Jasper Delaey[1], Sandra Van Vlierberghe[1]

[1] *Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281, S4-Bis, 9000 Ghent, Belgium*

Corresponding author's email: coralie.greant@ugent.be

Various kinds of breast defects exist for which the reconstruction remains a challenge. Conventional reconstruction techniques are accompanied with risks and complications such as infection, rupture, capsular contracture, donor site morbidity and uncontrollable resorption rates. Therefore, a strong research focus is directed towards regenerative adipose tissue engineering.

In the present work, a novel strategy is introduced to enable minimally invasive, patient-specific breast reconstructions. To this end, shape memory copolymers based on aliphatic polyesters were synthesized and functionalized into acrylate end-capped urethane-based polymers (AUPs) to enable chemical crosslinking after 3D-printing. The materials were characterized by NMR, DSC, TGA, GPC and mechanical characterization was performed to study the tensile strength and shape memory effect. The materials were tuned in order to achieve a shape shift right below body temperature. This shift in shape from a temporary reduced size into a permanent large volume, upon implantation in the body, assures the minimally invasive approach of the developed strategy.

Throughout the project, the AUPs were processed into porous scaffolds by means of additive manufacturing. The morphology of the obtained scaffolds was studied using SEM and micro-CT. The results indicated that stable scaffolds can be realized, with pore sizes ranging between 500µm and 1000µm. These structures were further characterized by performing mechanical and biodegradability assays and, after applying a cell-interactive gelatin-based coating, the effect of shape memory on cell behavior is currently being investigated.

As a result, the developed porous shape memory scaffolds can be considered promising candidates towards minimally invasive, patient-specific adipose tissue engineering.

Keywords

shape memory polymers; breast reconstruction; minimally invasive surgery

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Abstract **#223**

KIDNEY ORGANOID ENCAPSULATION IN SUPRAMOLECULAR HYDROGELS DIRECTS NEPHRON PROGENITOR LINEAGE COMMITMENT

Johnick van Sprangp[1], Patricia Dankers[1]

[1] *Eindhoven University of Technology*

Corresponding author's email: j.f.v.sprang@tue.nl

The differentiation of hiPSCs towards renal lineages allows for generation of tissue that highly resembles the mammalian kidney[1]. These hiPSC-derived tissues are known as kidney organoids and contain four renal progenitor cell populations, which develop into nephrons[2]. These kidney organoids hold translational promise as a regenerative therapy for patients suffering from renal failure[3]. However, their nephrons are not properly segmented, which may negatively impact their function[4]. The segmentation of nephrons is influenced by a proximal-distal gradient of Wnt- and Notch signaling, which determines the final identity of differentiating nephron progenitor cells[5]. These pathways are tuned by a wide array of cues, including mechanotransducive forces from the surrounding environment. Here, we take the first step towards investigating the role of biomaterials, and respective mechanical forces, on lineage commitment of nephron progenitor cells within organoids. To this end, kidney organoids were encapsulated in a fully synthetic hydrogel at different time points. These hydrogels are composed of UPy-based molecules, which are capable of self-assembling into a dynamic, fiber-like network via supramolecular interactions. Furthermore, by coupling integrin-binding peptides to UPy-moieties, it is possible to biofunctionalize the network to allow mechanotransduction. These properties together result in a fully synthetic hydrogel in which kidney organoids remain viable and show nephrogenesis. Furthermore, encapsulation of kidney organoids within UPy-hydrogels shifts the segmentation of nephrons towards the glomerular phenotype, irrespective of encapsulation timing. This demonstrates that the lineage commitment of nephron progenitor cells is susceptible to changes in the surrounding mechanical environment and may be directed in this fashion.

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Abstract **#224**

TANNIC ACID-MEDIATED E7/P15 PEPTIDE-FUNCTIONALIZED SCAFFOLD FOR ENDOGENOUS OSTEOCHONDRAL TISSUE REGENERATION

Wei Zhang[P][1]

[1] *Southeast University*

Corresponding author's email: zhang.wei@seu.edu.cn

A leading strategy in osteochondral tissue engineering is the development of cell-instructive scaffolds that provide biochemical cues to direct the recruitment of endogenous bone marrow mesenchymal stem cells (BMSCs), as well as to promote osteogenic and chondrogenic differentiation to stimulate osteochondral regeneration. In this study, we develop a novel peptide-functionalized alginate scaffold by prime-coating the scaffold with tannic acid (TA) followed by conjugation of E7 and P15 peptides. TA coating significantly enhanced the scaffold stability and mechanical properties, increased peptide conjugation rate and maintained the controlled release of peptides without affecting their bioactivity, in a concentration-dependent manner. Due to the conjugation of E7/P15 peptides, the modified alginate-TA scaffold showed satisfactory biocompatibility, significant improvement of BMSCs migration and deposition of cartilage and bone extracellular matrix (ECM) in vitro, as compared to the scaffold without peptide conjugation. The scaffold also simultaneously improved the regeneration of cartilage and subchondral bone when implanted into rabbit osteochondral defect. These findings thus suggest that the developed TA-mediated E7/P15 peptide-functionalized alginate scaffold has great potential to be utilized as a cell-instructive scaffold for osteochondral regeneration.

Abstract **#226**

IMPACT OF STEM CELLS ON WOUND HEALING AND INTEGRATION OF TISSUE ENGINEERED ALVEOLAR BONE

Hanna Malyaran[P][1,2], Kai Gerlach[1,2], Michael Wolf[3], Sabine Neuss[1,2]

[1] *Institute of Pathology, RWTH Aachen University Hospital*

[2] *Helmholtz Institute for Biomedical Engineering, Biointerface Laboratory, RWTH Aachen University*

[3] *Department of Orthodontics, Dental Clinic, RWTH Aachen University Hospital*

Corresponding author's email: hmalyaran@ukaachen.de

Decades of clinical experience indicate, that velocity and efficiency of wound healing and bone remodeling in alveolar bone is in part dependent on the exact localisation of the wound. In the maxilla, the upper jaw, these processes are generally faster and more efficient than in the mandible, the lower jaw. Alveolar bone differs in composition, with 23% bone marrow and 46% lamellar bone in the upper jaw, and 16% bone marrow and 63% lamellar bone in the lower jaw. The periodontal ligament (PDL) hosts endogenous stem cells, which were shown to differentiate towards osteoblasts and cementoblasts in 1976. Since 2004, specific PDL cells can be isolated from extracted third molars, demonstrating self-renewal and differentiation capacity towards several mesodermal cell fates and therefore being referred to as stem cells. Human mesenchymal stem cells (MSC) are beneficial for wound healing and tissue regeneration of mesodermal tissue. Periodontal ligament (PDL) cells can be isolated from third molars of the upper and lower jaw. Due to their stem cell like morphology and behavior, they can be compared with human MSC. PDL cells and in particular MSC have been extensively studied with respect to bone replacement strategies. The characterisation of PDL cells from the upper and lower jaw compared to MSC will identify similarities and differences in cellular composition and unravel molecular pathways involved in differentially regulated wound healing in mandible and maxilla.

Keywords

Periodontal Ligament Cells; Alveolar bone; Bone remodeling

Abstract **#227**

A REVERSIBLY-SEALED THYROID-ON-CHIP FOR CONTINUOUS MONITORING OF THYROID DISRUPTION

Daniel Carvalho$_P$[1]

[1] *MERLN*

Corresponding author's email: d.carvalho@maastrichtuniversity.nl

There is growing evidence that various environmental factors, such as plasticizers and pollutants, are Endocrine Disrupting Chemicals (EDCs), which are also associated to thyroid malfunctioning in the human body1,2. Currently, the safety of potential EDCs is poorly estimated due to the lack of physiologically relevant toxicological assays for preclinical testing. New, innovative 3D cell culture systems are urgently needed to accurately evaluate thyroid disruption, which can offer both automation and reduced labor for pharmaceutical companies. Moreover, integration of sensing technology has shown promise in precise assessment of the metabolic and functional parameters secreted by cells in response to drug compounds3. Here, we describe the first thyroid-on-chip based on a 3D polymeric membrane for dynamic culture of mouse thyroid follicles4. Cells were Matrigel-embedded on the microstructured membranes containing arrays of 200 thin-walled porous microwells with 350 µm in diameter. Membranes were reversibly sealed between elastomeric gaskets via mechanical clamps and fully integrated with optic sensors for continuous in situ monitoring of culture parameters. The clamping seal was found to withstand internal pressures higher than those obtained by irreversible plasma bonding while enabling re-opening of the chip without damage and direct access to cell culture inserts for downstream immunostaining. The 3D cultured thyroid cells survived up to 7 days and retained an in vivo-like organification characterized by luminal expression of thyroglobulin. We believe that this system has shown how organ-on-chip, sensing and clamping technologies may be leveraged to create next-generation in vitro cell-based assays for EDC screening.

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Abstract **#228**

LOCALIZED DELIVERY OF BIOMOLECULES USING THERMOSENSITIVE INJECTABLE HYDROGELS FOR THE TREATMENT OF CHRONIC SKIN WOUNDS

Arianna Grivet Brancot[P][1], Monica Boffito[1,2], Susanna Sartori[1], Simona Bronco[2], Gianluca Ciardelli[1,2]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129, Torino (Italy)*

[2] *Istituto per i processi Chimico-Fisici, Consiglio Nazionale delle Ricerche, Via G. Moruzzi, 1, 56124, Pisa (Italy)*

Corresponding author's email: arianna.grivet@polito.it

Chronic skin wounds (CSWs) are a worldwide healthcare problem, with a strong effect on patients' life and mortality risk, and an important impact on healthcare systems. Innovative treatments are thus needed to induce better tissue repair/regeneration and to improve patients' life quality. In this work, injectable thermosensitive hydrogels based on amphiphilic polyurethanes have been developed as drugs (e.g., ibuprofen) and biomolecules (e.g., platelet lysate) carriers in the treatment of CSWs.

Two poly(ether urethane)s (PEUs) were synthesized starting from commercial Poloxamer 407 [1], 1,6-diisocyanatehexane and different chain extenders (i.e., N-Boc serinol and L-lysine ethyl ester) PEUs aqueous solutions at appropriate concentrations (10-15% w/v) presented a sol-gel transition around 30-37°C after few minutes. The formulations showed finely tunable mechanical properties and residence time in aqueous environment modulated by PEU concentration, as assessed through rheological and stability tests. Injectability through medical needles and at different temperatures was also proved. The loading of model proteins (bovine serum albumin, BSA, and horseradish peroxidase, HRP) within the gels did not affect gelation and rheological properties. Payload release was completed between 7-14 days, depending on PEU concentration. Moreover, hydrogel ability to preserve the biological functionality of the encapsulated biomolecules was demonstrated by testing the HRP residual activity. Similar results were obtained regarding platelet lysate (PL) encapsulation and release.

The presented hydrogel formulations thus represent a promising tool for localized drug/biomolecule delivery in CSWs treatment, given their tunable gelation properties, residence time in aqueous environment and ability to encapsulate and release biomolecules with a sustained and controlled kinetics.

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Abstract **#229**

HYDROXYAPATITE-DECORATED FMOC-HYDROGEL AS A BONE MIMICKING SUBSTRATE FOR OSTEOCLAST DIFFERENTIATION AND CULTURE

Mattia Vitale$_P$[1], Bethan McAvan[1], Chris Allan[2], Stephen Michael Richardson[1], Judith Alison Hoyland[1]

[1] *Division of Cell Matrix Biology & Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester M13 9PT*

[2] *Biogelx Ltd - BioCity Scotland, Bo'Ness Rd, Newhouse, Chapelhall, Motherwell ML1 5UH*

Corresponding author's email: mattia.vitale@manchester.ac.uk

Hydrogels are biomaterials that recapitulate many of the extracellular matrix properties in tissue engineering applications. However, their use in bone regeneration is limited. Furthermore, most studies utilising hydrogels for bone tissue engineering applications are predominantly focused on osteoblasts and bone formation, while osteoclasts are often overlooked. Yet, the osteoclast role is pivotal for bone homeostasis and aberrant osteoclast activity also is involved in several pathological diseases such as cancer. Thus, we aim to develop customised hydrogels for use as substrates for osteoclast differentiation and culture. We present here new data on a composite hydrogel based on the self-assembling gelator peptides Fmoc diphenylalanine/serine (Fmoc-FF/S) and Fmoc-RGD, which has been modified by incorporation of hydroxyapatite nanopowder (HAp). This new hydrogel shows improved mechanical properties and supports osteoclast adhesion and differentiation. Atomic Force Microscopy and Rheological analysis of the HAp-decorated hydrogels showed a larger fibre network diameter and higher mechanical strength than HAp-free hydrogels, with a doubled storage modulus. Additionally, staining for F-Actin showed that the hydrogels supported cell adhesion and cell viability in vitro. Interestingly, HAp-decorated hydrogels were able to support osteoclastogenesis in vitro as Raw 264.7 cells showed typical morphology of mature osteoclasts such as presence of multinuclei and an actin ring. Moreover, the presence of TRAP-positive granules suggests the differentiation of Raw 264.7 cells into mature osteoclasts. These results suggest that the HAp-decorated hydrogels developed here could be the basis for building a more complex and realistic biomaterial for differentiation and culture of osteoclasts and ultimately improved bone regeneration.

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Abstract **#230**

DESIGN OF POLY(ESTER URETHANE)S TO ENGINEER BIOCOMPATIBLE 3D-PRINTED SCAFFOLDS FOR CARDIAC TISSUE REPAIR AND REGENERATION

Arianna Grivet Brancot[P][1,2], Monica Boffito[1], Gianluca Ciardelli[1]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Corso Duca degli Abruzzi, 24, 10129, Torino (Italy)*

[2] *Department of Surgical Sciences, Università di Torino, Corso Dogliotti 14, 10126, Torino (Italy)*

Corresponding author's email: arianna.grivet@polito.it

Myocardial infarction, a common occurrence for European and North American population, can result in severe cardiac tissue damage that cannot be successfully compensated by current therapies and surgical methods. Thus, in recent years many studies have been focused on patches able to support the impaired tissue and induce regeneration through cardiac progenitor cell attachment, proliferation and differentiation using appropriate stimuli.

In this contribution, a library of poly(ester urethane)s (PUs) based on poly(ε-caprolactone), 1,6-diisocyanatohexane and aliphatic chain extenders with different chain length (i.e., 1,4-butanediol, 1,8-octanediol, 1,12-dodecandiol) has been developed and characterized. Size Exclusion Chromatography evidenced a number average molecular weight comprised between 30 and 40 kDa for all the synthesized PUs. The thermal and thermomechanical properties obtained through thermogravimetric analysis, Differential Scanning Calorimetry and rheological characterization demonstrated PU suitability to processing through common Fused Deposition Modeling (FDM) printers. This opens to the possibility to develop personalized patches that can be designed to finely reproduce the patient's specific defect. To improve the hydrophilicity of the construct surface, a plasma treatment using allylamine as monomer was optimized to expose –NH2 groups, resulting in a significant decrease in the contact angle values. The exposed amino groups on the surface were then exploited to graft proteins (e.g., laminin-1, collagen) and peptides (e.g., RGD) on the scaffolds to increase biomimicry and cell attachment [2]. Thus, these surface-modified materials show a good potential for employment in cardiac tissue engineering, with the aim to successfully stimulate tissue repair and promote myocardial regeneration toward a fully functional heart recovery.

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Abstract **#231**

ENGINEERING HUMAN VASCULARIZED IMMUNO BONE MINITISSUES AS ANTIMETASTATIC DRUG SCREENING PLATFORMS

Maria Vittoria Colombo[P][1,2], Simone Bersini[1], Chiara Arrigoni[1], Mara Gilardi[3], Veronica Sansoni[4], Giovanni Lombardi[4], Gabriele Candiani[2], Matteo Moretti[1,3]

[1] *Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Lugano (CH)*

[2] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano (IT)*

[3] *Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milano (IT)*

[4] *Laboratory of Experimental Biochemistry and Molecular Biology, I.R.C.C.S. Istituto Ortopedico Galeazzi, Milano (IT)*

Corresponding author's email: mariavittoria.colombo@polimi.it

The discovery of new drugs against breast cancer metastases is a primary goal for research and pharma industry; however, current in vitro and in vivo models oversimplify breast cancer cells (bCCs) interactions with the bone microenvironment or present species-specific differences in biological mechanisms, respectively [1]. To overcome these limitations, based on our previous work [2,3], we embedded up to five human cell types (i.e. osteoblasts, osteoclasts, endothelial cells, macrophages, bCCs) in a 3D fibrin gel model developing a highly complex vascularized metastatic immuno bone minitissue (MIBm). The MIBm closely mimics biological processes seen in vivo as the organ-specific proliferation of bCCs, with respect to other tissue that are not affected by breast cancer metastasis as muscle, and the bCC acquisition of osteomimicry properties. Moreover, the macrophages activation towards M2 phenotype, which are immunosuppressive favoring tumor growth, and the alteration of vascular structure suggest that our minitissue is able to reproduce some key features of the early metastatic niche. We treated MIBm and a bCC monoculture minitissue with rapamycin and doxorubicin, two FDA approved drugs. The dose required to impair bCC growth was significantly higher in MIBm than in the bCC monoculture minitissue, in addition both drugs had an anti-angiogenic effect. In this study [4], we showed that the drug effects were strictly dependent on the presence of the bone-tumor microenvironment and we could investigate drug influence on other cell components suggesting that the MIBm could better perform antimetastatic drug screening than more simplistic models.

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Abstract **#232**

DEVELOPMENT OF STABLE THERMORESPONSIVE MULTILAYERS BASED ON CROSS-LINKED PNIPAM-GREAFTED-CHITOSAN AND HEPARIN FOR APPLICATION IN TISSUE ENGINEERING

Yi-Tung Lu[P][1], Kui Zeng[2], Bodo Fuhrmann[3], Kai Zhang[2], Thomas Groth[1,3]

[1] Department of Biomedical Materials Science and Engineering, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Heinrich-Damerow-Strasse 4, 06120, Halle (Saale), Germany

[2] Department of Wood Technology and Wood Chemistry, Georg-August-Universität Göttingen, Büsgenweg 4, D-37077 Göttingen, Germany

[3] Interdisciplinary Center of Material Research and Interdisciplinary Center of Applied Research, Martin Luther University Halle-Wittenberg, 06099, Halle (Saale), Germany

Corresponding author's email: yi-tung.lu@student.uni-halle.de

The thermoresponsive poly (N-isopropylacrylamide) (PNIPAM) exhibits a lower critical solution temperature (LCST) at 32°C allowing cell detachment by simply decreasing temperature below LCST. Here, layer-by-layer technique has been used to develop thermoresponsive polyelectrolyte multilayer (PNIPAM-PEM) systems that shall be applied for fabrication of cell sheets. Chitosan is covalently grafted with PNIPAM (PNIPAM-CHI) to obtain different derivatization degrees and used as polycation. Heparin is used as polyanion. The degrees of substitution (DS) and thermoresponsive properties of PNIPAM grafted onto chitosan are analyzed by 1H NMR and dynamic light scattering. PNIPAM-CHI with different DS are obtained and higher DS showed significant change in size above LCST. The growth of PNIPAM-PEMs composed of PNIPAM-CHI and heparin fabricated at pH 4 are studied by surface plasmon resonance. PNIPAM-CHI with different DS represent similar linear growth behavior to form multilayers with heparin. Crosslinking of PNIPAM-PEM using EDC/NHS is conducted to enhance stability. The thickness of multilayers measured by ellipsometry show that non-crosslinked and crosslinked systems are not different. However, the thickness decreases significantly in non-crosslinked system after rinsing with phosphate buffer, pH 7.4 compared to negligible changes in the crosslinked multilayers. The applicability of PNIPAM-PEMs as cell culture substrate is examined using multipotent mouse stem cells. Higher cell adhesion on crosslinking system after 24 h cultivation is observed in agreement with the stable thickness and complete multilayer formation. Therefore, the PNIPAM-PEMs with cross-linking provides greater stability as a cell culture system for further investigation of cell sheet generation for application in tissue engineering.

Keywords

thermoresponsive; polysaccharide; Layer-by-layer technique

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Abstract **#233**

ANGIOGENIC POTENTIAL OF SENESCENT MESENCHYMAL STROMAL CELLS

Andrey Ratushnyy[1], Mariia Ezdakova[1], Ludmila Buravkova[1]
[1] *Institute of Biomedical Problems, Russian Academy of Science*
Corresponding author's email: ratushkin@mail.ru

The mesenchymal stem/stromal cell (MSC) secretome influences the microenvironment upon injury, promoting cytoprotection, and tissue repair of the damaged area. These effects are of particular interest for the treatment of ischemic damaged tissues via angiogenesis promoting. MSC senescence results in secretome modulation (senescence associated secretory phenotype - SASP), which affects the neighboring cell functions.
MSCs were isolated from human adipose tissue. Long-term cultivation was continued until replicative senescence (19-28 passages). Senescence of MSCs was identified by assessing the activity of β-galactosidase, population doubling (PD) per passage, cell morphology etc. Conditioned medium (CM) was collected for further analysis, including chorioallantoic membrane assay in ovo, capillary-like tube formation on Matrigel, cell migration assay, analysis of MSC secreted proteins. Total RNA was extracted from MSCs for qPCR analysis.
Angiogenic potential of senescent MSC CM was attenuated which was characterized by decreased number of blood vessels in chorioallantoic membrane assay in ovo. In the same time long-term cultivation did not affect endothelium cell capillary-like network of tubule complexes on Matrigel and non-directed endothelium cell migration in vitro. Analysis of CM had shown increased concentration a number of cytokines (MCP-3, IL-4, IFN-a2, MDC, IP-10, FGF-2, RANTES, GRO, IL-8, IL-6, MCP-1) with pro- and antiangiogenic activity. PCR analysis revealed both down- and upregulation of some angiogenesis-associated genes.
Thus, modulations of MSC secretory phenotype under senescence result in decreased angiogenic activity in complex model in ovo, but not on Matrigel with endothelium cells only.
This study was supported by the RFBR (project no. 19-015-00150).

Abstract **#234**

MICRORNA-RELEASING LIPOPLEXES IN THE DIRECT REPROGRAMMING OF ADULT HUMAN CARDIAC FIBROBLASTS INTO CARDIOMYOCYTES

Letizia Nicoletti[1,2,3], Giulia Tarricone[1,2,3], Camilla Paoletti[1,2,3], Carla Divieto[4], Ilaria Andreana[5], Barbara Stella[5], Silvia Arpicco[5], Clara Mattu[1,2,3], Valeria Chiono[1,2,3]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Turin, Italy*

[3] *POLITO Biomedlab, Politecnico di Torino, Turin, Italy*

[4] *Division of Metrology for Quality of Life, Istituto Nazionale di Ricerca Metrologica, Turin, Italy*

[5] *Department of Drug Science and Technology, University of Turin, Turin, Italy*

Corresponding author's email: letizia.nicoletti@polito.it

Myocardial infarction causes the irreversible loss of cardiomyocytes and the formation of a non-contractile fibrotic scar. Among advanced therapies for myocardial regeneration, in situ release of specific microRNAs (miRNAs) is under study to promote cardiomyocyte proliferation or transdifferentiation of cardiac fibroblasts (CFs) into induced cardiomyocytes (iCMs)[1,2]. Our group has demonstrated that a combination of miRNAs (miRcombo: miR-1, 133, 208, 499), previously identified on mouse model, can trigger direct reprogramming of human CFs into iCMs[2]. In this work, new lipoplexes based on a mixture of cationic and helper lipid were designed for more efficient encapsulation and release of miRNAs to human CFs respect to commercial agents, enhancing direct reprogramming efficiency. Lipoplexes encapsulating negmiR or miR-1 were initially prepared at 3.0 to 0.35 N:P ratios, showing 99% encapsulation efficiency, and average hydrodynamic size and zeta potential respectively ranging from 372 nm to 876 nm and from +40 mV to -26 mV with decreasing N:P ratio. Based on stability experiments in different media at different temperatures (4°C and 37°C), lipoplexes with 3 N:P ratio were selected for in vitro tests with human CFs, showing biocompatibility and efficient miR-1 release to CFs. Then, miRcombo-loaded lipoplexes were characterized for their physicochemical and biological properties, triggering enhanced cardiac markers expression at 15 days compared to commercial transfection agents. A shear-thinning alginate hydrogel is under development for intramyocardial injection and localized release of lipoplexes.

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Abstract **#235**

PHYSICAL AND BIOLOGICAL CHARACTERISATION OF MECHANICALLY AND STRUCTURALLY MIMETIC SMALL CALIBRE VASCULAR SCAFFOLDS

Risto Martin[P][1], Pierre-Alexis Mouthuy[2], Sarah Waters[3], Hua Ye[1]

[1] *Department of Engineering Science, Institute of Biomedical Engineering, University of Oxford, Oxford, UK*

[2] *Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Botnar Institute of Musculoskeletal Sciences, University of Oxford, Oxford, UK*

[3] *Mathematical Institute, University of Oxford, Oxford, UK*

Corresponding author's email: risto.martin@lincoln.ox.ac.uk

Introduction: In part due to the mismatch of micro-topographical structure and mechanical properties between native blood vessels and their synthetic counterparts, small calibre vascular scaffolds (SCVSs) smaller than ø 6mm have failed to achieve clinical implementation[1]. To overcome these issues, a mechanical profile of native blood vessels was developed through systematic literature review. The profile then guided the manufacture of a biomimetic and biodegradable blended Polycaprolactone and Polydioxanone electrospun SCVS.

Methods: SCVSs were continuously electrospun via a bespoke rotating wire collector[2], with different polymer and pyridine concentrations. From scanning electron microscopy images of the SCVSs micro-topographical characteristics were quantified with FIJI-DiameterJ. The SCVSs were mechanically and structurally profiled using uniaxial tensile testing, microCT, mercury porosimetry, and gravimetric analysis. To aid translation, biocompatibility was evaluated in line with ISO 10993-5:2009.

Results: Varying gross polymer and pyridine concentrations between 0.0925 – 0.193 g/ml and 0.1 – 5 PPM altered the micro-topography, porosity, shape, and mechanical properties of the SCVS. Between these concentrations, microfibre diameter and the Young's modulus ranged between 0.9 – 1.55 µm and 2.09 – 11.45 MPa versus 0.22 – 2.61 MPa for native blood vessels. Additionally, the SCVS had a porosity, pore diameter, lumen diameter and wall thickness of 70 – 85%, 4 ± 1 µm, 100 ± 25 µm, and 300 ± 100 µm respectively.

Conclusion: Successful translation of SCVSs relies on mechanical and micro-topographical mimeticism. For the first time, SCVSs were continuously electrospun and demonstrated to have microstructure and elasticity comparable to that of native blood vessels.

Keywords

Advanced materials; Vascular scaffold; Electrospinning

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Abstract **#236**

3D NANOGROOVED LIVING PLATFORMS FOR THE AUTONOMOUS DEVELOPMENT OF VASCULARISED BONE TISSUE CONSTRUCTS

Isabel Marinho Bjørgeᵖ[1], Clara R. Correia[1], João F. Mano[1]

[1] *Department of Chemistry, CICECO, University of Aveiro, Portugal*

Corresponding author's email: isabelbjorge@ua.pt

The influence of surface topography on the differentiation ability of stromal cells has been established in 2D yet is lacking in tissue mimetic 3D-environments. We recently proposed nanogrooved microdiscs (topodiscs) for a bottom-up cell-mediated 3D fabrication, which remarkably resulted in osteogenic differentiation of adipose-derived stromal cells (ASCs) even in basal medium [1]. Inspired in the multiphenotypic environment of bone and taking advantage of the liquefied-core capsule technology [2], 3D-preaggregated topodiscs and ASCs were co-encapsulated with endothelial cells (ECs). Uniform ASC-topodisc microaggregates were encapsulated within an alginate core containing dispersed ECs, enveloped by a permselective membrane. Upon mild core liquefaction, a sequential seeding is established and ASCs and ECs are able to crosstalk directly. By combining topographical cues with cell signalling pathways, we aimed to develop prevascularised bone-like microtissues in an autonomous fashion. Results show that ASC-topodisc microaggregates proved to be optimal supports for EC adhesion, further promoted using bioreactors to create dynamic culture conditions. Successful biological activity was confirmed via increased DNA content and the merging of microaggregates into macro 3D constructs. At 21 days post-encapsulation with topodiscs, ASCs differentiated towards the osteogenic lineage in static and dynamic conditions for both mono and co-cultures as verified by the presence of hydroxyapatite and osteopontin, even without osteoinductive factors. This was not observed in monoculture conditions featuring spherical or non-grooved disc particles, highlighting topodiscs' nanogrooves as an impacting factor to induce osteogenesis. Next, we plan to assess the neo-vascularisation of the established co-culture, and the quality of the bone-like microtissue formed.

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Abstract **#237**

IN-SILICO MODELLING AS A TOOL TO RECAPITULATE THE NUTRIENT MICROENVIRONMENT OF THE INTERVERTEBRAL DISC FOR THE DEVELOPMENT OF CELL THERAPIES

Emily McDonnell[P][1]

[1] *Trinity College Dublin*

Corresponding author's email: mcdonne5@tcd.ie

The intervertebral disc is a large avascular structure with a microenvironment characterised by low nutrient concentrations and increased acidity.1,2 A number of in silico models have been developed to provide valuable insights into disc nutrient transport and metabolism.3 However, their reliability is limited by their experimental input parameters. To optimise physiologically relevant culturing conditions, this work developed computational models to predict the metabolite gradients that develop during in vitro and ex vivo organ culture and compared them to in vivo models' nutrient concentrations.

Finite element models governed by coupled reaction-diffusion equations were created using COMSOL Multiphysics. Metabolic rates were dependent on local oxygen and pH by employing equations derived previously.1,4 Results highlighted limitations in cell density parameters most typically used in predictive modelling.5 We also experimentally validated these predictive models within ex vivo disc cultures using microprobe measurements and biochemical assays. Furthermore, by incorporating a viability criterion, predicted pH and oxygen show better agreement with in vivo disc measurements.2,7

This highlights that more robust cell parameters are needed to improve the accuracy of in silico models and that in vitro and ex vivo culturing regimes need to be refined to more closely represent the typical nutrient microenvironments found in vivo.

Keywords

nutrient microenvironment; culturing conditions; optimisation

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Abstract **#238**

ELUCIDATION OF THE DYNAMICS OF MESENCHYMAL STROMAL CELL-MEDIATED ENDOCHONDRAL OSSIFICATION IN A SUBCUTANEOUS IMPLANTATION MODEL

Andrea Lolli[P][1], Niamh Fahy[1,2], Janneke Witte-Bouma[1], Virginia Palomares Cabeza[1,3], Eric Farrell[1]

[1] *Department of Oral and Maxillofacial Surgery, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

[2] *Department of Orthopaedics, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

[3] *School of Veterinary Medicine, University College Dublin, Dublin, Ireland*

Corresponding author's email: a.lolli@erasmusmc.nl

Endochondral ossification (EO) is the process by which bones are formed, via a cartilage template. We previously demonstrated that chondrogenically-primed human mesenchymal stromal cells (hMSCs) implanted subcutaneously in mice generate bone via EO(1). To understand how best to control the process and ultimately develop strategies for bone repair, we investigated the kinetics of hMSC-mediated bone formation in vivo, which still remain elusive.

Bone marrow-derived hMSCs were cultured as pellets with the chondrogenic inducer Transforming Growth Factor(TGF)-β3 for 7 ("P7") or 21 days ("P21"), or without TGF-β3 for 7 days ("p7"), and implanted subcutaneously in BALB/c athymic mice (2 hMSC donors, 30 mice/donor). While p7 pellets were rapidly resorbed, P7 and P21 pellets were encapsulated by fibrous tissue (d3-d7), underwent mineralization (d14) and microvascular infiltration (d28), forming bone with marrow (d56-d84). Human-specific GAPDH staining proved that human cells persisted until d84. Micro-computed tomography demonstrated increased bone volume for P21 pellets (5-fold), while the bone density was comparable to P7 pellets. Flow cytometry analysis of P7 and P21 pellets indicated consistent presence of CD11b+Ly6G+CD115- neutrophils and CD11b+F4/80+ macrophages from d3 onwards. Characterization of macrophage subsets revealed donor-specific patterns in the expression of pro-inflammatory (CD86), tissue repair (CD206) and anti-inflammatory (CD163) markers.

We provide the first longitudinal analysis of hMSC-mediated EO in vivo. The amount/maturity of the implanted cartilage impacts bone formation, possibly due to differential involvement of innate immune cells, which are rapidly recruited and persist during bone formation. These data lay foundations for new "developmentally-inspired" tissue engineering approaches for bone defects.

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Abstract **#239**

THIOUREA-CATECHOL COUPLING CHEMISTRY ENABLING THE DEVELOPMENT AND FUNCTIONALIZATION OF CYTOCOMPATIBLE HYDROGEL ADHESIVES

Kongchang Wei[P][1]

[1] *Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biomimetic Membranes and Textiles*

Corresponding author's email: kongchang.wei@empa.ch

Inspired by marine mussels, polymers are typically functionalized with catechol moieties via nonreactive amides or ester linkages, thereafter, the formation of mussel-inspired hydrogels can be induced by in situ oxidation.[1,2] However, with nonreactive linkages, the catechol-functionalized polymers usually need a long cross-linking period (minutes) under basic conditions. Although using strong oxidants such as sodium periodate (NaIO4) could shorten the time, the harsh condition could limit their application in cell delivery.[3] Recent investigations on mussel adhesion have revealed that, besides catechol-rich Mfps, thiol-rich Mfp-6 also plays an important role in the remarkable adhesion of mussel.[4] In this contribution, I will present a dual-mode-mimicking strategy by modifying polymers with thiourea (TU) and catechol (Cat) functionalities to mimic two types of Mfps, namely cate-chol-rich Mfps and thiol-rich Mfps, respectively.[5-8] The TU-Cat coupling chemistry can be used not only for rapid polymer crosslinking, but also for effective fuctionalization of 2D substrates or 3D matrix. The reaction can be catalysized by anzymes under mild conditions in a pH-independent manner, thus showing great potential in novel biomaterials development and bioactivation.

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Abstract **#240**

A DEVELOPMENTAL TISSUE ENGINEERING APPROACH TO GENERATE BONE CONSTRUCTS FOR THE STUDY OF BONE METASTASIS

Andrea Lolli[P][1], Janneke Witte-Bouma[1], Blake Tutterow[1,2], Massimo Saini[3], Nicola Aceto[3], Eric Farrell[1]

[1] *Department of Oral and Maxillofacial Surgery, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

[2] *Regenerative Medicine Institute (REMEDI), National University of Ireland Galway, Galway, Ireland*

[3] *Department of Biomedicine, Cancer Metastasis Lab, University of Basel and University Hospital Basel, Basel, Switzerland*

Corresponding author's email: a.lolli@erasmusmc.nl

Breast cancer metastasis affects primarily the bones, causing severe symptoms including pain and fractures, and high mortality rate. Unfortunately, the mechanisms and vulnerabilities of bone metastasis are still elusive, and the patients remain incurable. Taking inspiration from endochondral ossification, the natural process whereby bones are formed via a cartilage template, we aimed to develop a new strategy to generate bone constructs in vivo and in vitro for studying bone metastasis.

Bone marrow-derived human mesenchymal stromal cells were chondrogenically differentiated as pellets for 21 days to induce cartilage production, and implanted subcutaneously in athymic mice for 8 weeks. Micro-computed tomography and histological analysis demonstrated that the constructs underwent endochondral ossification forming vascularized bone with marrow. To generate in vitro constructs, chondrogenic pellets were exposed to osteogenic conditions (β-glycerophosphate), inducing cartilage mineralization. In transwell co-cultures, in vivo and in vitro-derived constructs enhanced the migration of human metastatic breast cancer cells (MDA-MB-231) (12-fold and 36-fold, respectively), but did not affect non-metatastic cells (MCF7). Preliminary experiments with exposure of MDA-MB-231 to conditioned medium from mineralized pellets showed enhanced cancer cell proliferation and migration.

We exploited a developmental tissue engineering approach to generate bone constructs for studying aspects of bone metastasis. In vivo- and in vitro-generated bone/mineralized constructs were successfully co-cultured with invasive breast cancer cells, inducing a migratory response. These experimental models will be applied for studying the molecular basis of metastasis and developing new targeted drugs.

Abstract **#241**

A 3D PRINTABLE CEMENT FOR BONE REGENERATION

Marie-Michèle Germaini[P1], Marie-Michèle Germaini[1,2], Pierre Weiss[1,3], Sofiane Belhabib[4], Sofiane Guessasma[5], Joëlle Veziers[1,3], Steven Nedellec[6], Hélène Gautier[1,7], Boris Halgand[3,1], Benoit Furet[8], Remi Deterre[4], Thierry Rouillon[9,1], Pierre Corre[2,1]

[1] *Inserm UMR 1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS, Nantes, F-44042, France*

[2] *CHU Nantes, service chirurgie maxillo-faciale et stomatologie, PHU4 OTONN, Nantes, F-44093, France*

[3] *CHU Nantes, PHU4 OTONN, Nantes, F-44093, France*

[4] *Laboratoire GEPEA, UMR CNRS 6144, université-IUT de Nantes, avenue du Professeur Jean Rouxel, 44475 Carquefou Cédex, France*

[5] *INRA, UR1268 Biopolymères Interactions Assemblages, F-44300 Nantes, France*

[6] *INSERM UMS 016, SFR François Bonamy, MicroPicell Core Facility, Nantes, France*

[7] *Université de Nantes, UFR sciences biologiques et pharmaceutiques, Nantes, F-44093, France*

[8] *IMT Atlantique, 4 rue Alfred-Kastler, Nantes 44307, Le laboratoire des sciences du Numérique de Nantes (LS2N)*

[9] *Université de Nantes, UFR Odontologie, Nantes, F-44042, France*

Corresponding author's email: marie-michele.germaini@univ-nantes.fr

Bone is a dynamic tissue that can elself renewal. In some case regarding critical size and pathophysiological state of the bone defect a bone substitute is required. Different biomaterials can be used as pristine injectable biomaterials, granules, dense or porous blocks combined with autologous bone or bioactive molecules. The 3D printing technology has attracted keen attention especially because of the boom of affordable 3D printers and the different advantages offered by this technic namely the customization of the external shape of a bone defect and the design of a suitable interconnectivity porosity for enhancing healing-performance. A 3D printable phosphocalcic cement-based formulation has been developed. Alpha tricalcium phosphate the precursor of the calcium deficient apatite cement was blended with a physical gel of hyaluronic acid. The rheological properties demonstrated specific properties required for a printable formulation: shear-thinning, self-healing, thixotropic, when compared to the pristine cement.

This formulation can be extruded with the pneumatic pression compatible with the marketed printers. The self-hardening of the formulation is compatible with the time of printing and avoide time-consuming steps such as debinding and sintering used for others 3D printing processes.

Mechanical characterizations have shown a higher deformation rate for the composite formulation compared to the pristine cement.

Biological evaluations have validated biocompatibility of the formulation, ability to promote cell adhesion and proliferation.

In vivo experiments are planned for assessing the healing-performance of the formulation.

Keywords

bone regeneration; 3D printing; phosphocalcic cement

Abstract **#242**

UHEART: A BEATING HEART-ON-CHIP FOR GENERATION OF FUNCTIONAL 3D CARDIAC MICROTISSUES AND DIRECT ON-LINE ELECTROPHYSIOLOGICAL RECORDING

Roberta Visone[P][1,2], Giovanni Stefano Ugolini[1], Simona Marzorati[3], Enrico Pesenti[3], Paola Occhetta[1,2], Marco Rasponi[1]

[1] *Politecnico di Milano*

[2] *BiomimX Srl*

[3] *Accelera Srl*

Corresponding author's email: roberta.visone@polimi.it

Modelling the human cardiac pathophysiology in vitro represents a breakthrough to investigate key biological aspects of cardiovascular diseases progression. Organs-on-chip provide unprecedented opportunities to recreate tissue-specific microenvironments and to analyze functional readouts. Here we present uHeart, a beating heart-on-chip integrating i) uBeat technology[1], providing microtissues with mechanical stimulation resembling heart-beating and ii) micro-Electrode Channel Guide (µECG) technology, to extract electrophysiological signals on-line. We exploited uHeart to develop 3D in vitro cardiac models and we demonstrated its efficacy in screening drug cardiotoxicity.

Human induced pluripotent stem cell derived cardiomyocytes and dermal fibroblasts were embedded in fibrin gel in a 3:1 ratio[2] and mechanically trained through uBeat (i.e. 10%stretching, 1Hz). Microtissues' functionality and electrophysiological properties (i.e. beating period, spike amplitude-SA, field potential duration-FPD) were measured on-line by positioning electrodes through µECG and by performing gene expression analysis on ion-channels related genes. Finally, drug screening was conducted using three calibration drugs (i.e. Aspirin, Sotalol, Verapamil) and one vehicle (i.e. DMSO).

After 7 days in uHeart, cardiomyocytes exhibited sarcomeres' striations and beat spontaneously as a syncytium (frequency:0.59±0.12Hz, FPD:0.6±0.2s, SA:590±440µV). uBeat contributed to upregulate potassium channel related genes (i.e. h-ERG, IKs), responsible for the cardiac repolarization phase. uHeart efficiently captured the FPD alterations caused by compounds: DMSO and Aspirin did not alter the FPD, while Sotalol (15µM) and Verapamil (50nM) prolonged and shortened the repolarization phase, respectively, according to previous studies[3].

uHeart for the first time successfully combines mechanical stimulation of microtissues with on-line electrophysiology evaluation on-chip, offering a promising tool for pathophysiological studies.

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Abstract **#244**

IN VIVO MODULATION OF ASTROCYTIC CELL POPULATIONS BY HUMAN BONE MARROW MESENCHYMAL STEM CELL SECRETOME

Jonas Campos$_P$[1,2,3], Sonia Guerra-Gomes[1,2], Sofia Serra[1,2], Graça Baltazar[3], João Oliveira[1,2], Fábio Teixeira[1,2], António Salgado[1,2]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal*

[2] *ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal*

[3] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*

Corresponding author's email: campos.jonas@hotmail.com

The central nervous system has a limited auto-regenerative capacity, imposing challenges for the development of new therapies. Previous studies from our lab have demonstrated the applicability of human bone marrow mesenchymal stem cells (hBM-MSCs) secretome, as a modulator of the hippocampal neurogenic niche. Astrocytes are glial cells that control the neurogenic niche through the secretion of trophic factors and neuromodulators. Thus, in the present work, we aimed to evaluate the modulatory impact of astrocyte signaling on the hippocampal neurogenic niche upon application of hBM-MSCs secretome, centering our analysis on niche-specific proliferative events and morphological responses of astrocytes. Hence, we employed both wild-type and dnSNARE mice, a transgenic model that presents a blockage of astrocytic exocytosis and function impairment. Animals received bilateral injections of hBM-MSCs secretome at 8 weeks of age and hippocampal slices were evaluated with immunohistochemistry assays. Results demonstrated increased hippocampal proliferation marked by the number of Ki-67 expressing cells in wild-type animals when treated with secretome. Additionally, we observed that dnSNARE animals injected with hBM-MSCs secretome disclosed increased proliferation of GFAP stained cells at the sub-granular zone which is indicative of a local proliferative response of radial glia and early neural progenitors. Morphometrical evaluation found increased process hypertrophy and branching of dnSNARE astrocytes when treated with secretome. These results are closely related with the trophic factors present in the secretome, but also demonstrate a mechanistic involvement of astrocyte exocytosis in the proliferative response conferred by the secretome, placing astrocytes as key cellular targets for CNS regenerative medicine approaches.

Abstract **#245**

ASSEMBLING AN ELECTROSPUN PCL CORD FOR LIGAMENT REPAIR USING A SEMI-INDUSTRIAL BRAIDING MACHINE

L. Savic$_P$[1], E. M. Augustyniak[1], A. J. Carr[1], A. Price[1], S. Snelling[1], P.-A. Mouthuy[1]

[1] *University of Oxford, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Botnar Research Centre*

Corresponding author's email: luka.savic@ndorms.ox.ac.uk

Anterior Cruciate Ligament (ACL) repair using synthetic materials still result in less-than-adequate clinical outcomes and is characterised by high failure rate, owing to their inadequate mechanical and biological properties (1). Consequently, there is a need to produce better solutions. While polycaprolactone (PCL) electrospun materials have showed potential for soft tissue repair, they have not yet been produced into the form of a braided cord that can be produced at large scale.

PCL filaments used for braiding were produced with continuous electrospinning on a metal wire (2). The raw filaments were stretched 7-8 times the starting length, before being carefully assembled in a 9-filament bundles, which were used for braiding. The mechanical properties of the braided patch and individual filaments were measured by uniaxial tensile machine. Scaffold morphology was assessed via scanning electron microscopy (SEM).

The SEM images showed a biomimetic morphology of the aligned microfibres similar to the ACL microstructure. The braided PCL patch, produced with 9 filaments per carrier and using a total of 24 carriers (216 filaments overall) had tensile strengths comparable to market competitor FiberWire®.

We have shown that continuous electrospun filaments made of PCL could be braided by means of a commercial machine. This is a noteworthy improvement in the field of utilitarian bioresorbable clinical materials, specifically for applications in soft tissue repair.

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Abstract **#246**

POTENT AUTOCRINE SIGNALING BY MESENCHYMAL CELLS EXPRESSING BMP-2

Aysegul Atasoy-Zeybek$_P$[1], Michael Coenen[1], Gresin Hawse[1], Delphine Logeart-Avramoglou[2], Chris H. Evans[1], Rodolfo E. De la Vega[1,3]

[1] *Musculoskeletal Gene Therapy Research Laboratory, Rehabilitation Medicine Research Center, Mayo Clinic, Rochester, MN USA*

[2] *CNRS 7052, INSERM U1271, Université de Paris, Ecole Nationale Vétérinaire d'Alfort, Paris, France*

[3] *Department cBITE, MERLN – Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands*

Corresponding author's email: Atasoy-Zeybek.Aysegul@mayo.edu

Recombinant human bone morphogenetic protein (rhBMP-2) is an osteogenic morphogen used for orthopedic applications; clinical results, however, are modest. While studying gene transfer to deliver BMP-2, we have consistently noted superior bone healing in animal models with transient expression of small amounts of transgenic BMP-2. This suggests that endogenously synthesized BMP-2 is more potent than rhBMP-2 for osteogenesis. Here we have compared the response of cells to exogenous rhBMP-2 against BMP-2 synthesized endogenously following transduction with recombinant adenovirus encoding BMP-2 (Ad.BMP-2). This study used stably transfected murine mesenchymal cells containing a BMP-2 response element fused to a luciferase reporter gene (1). Cells were exposed to rhBMP-2 or transduced with Ad.BMP-2. BMP-2 activity was challenged with noggin and the BMP receptor kinase inhibitor LDN-212854. Luciferase activity and immunofluorescence staining were performed to determine the BMP-2 response. Intra- and extracellular hBMP-2 concentrations were measured by ELISA. Signaling via P-smad1/5/8 was assessed by western blot. Sub-nanogram amounts of BMP-2 synthesized by transduced cells induced higher luciferase activity than even a supraphysiological dose of rhBMP2 (1000 ng/mL). This superior potency was confirmed by immunofluorescence. Media conditioned by transduced cells did not induce luciferase in naïve cultures, suggesting that the response to endogenously synthesized BMP-2 occurred in an autocrine fashion. This response occurred via p-smad signaling, was resistant to inhibition by noggin but was inhibited by LDN-212854. The existence of a highly efficient autocrine BMP-2 signaling pathway helps explain the superiority of gene delivery over protein delivery as a means of stimulating osteogenesis in vivo.

Keywords

BMP2 ; bone healing; adenovirus mediated gene therapy

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Abstract **#247**

3D NEGATIVE PRINTING: UNLOCKING MATERIAL VERSATILITY AND DESIGN COMPLEXITY FOR TISSUE ENGINEERING SCAFFOLDS

Stephanie Doyle[P][1,2], Cathal O'Connell[1,2], Serena Duchi[3,2], Carmine Onofrillo[3,2], Elena Pirogova[1], Claudia Di Bella[3,2]

[1] *RMIT University*

[2] *BioFab3D - St Vincent's Hospital Melbourne*

[3] *The University of Melbourne*

Corresponding author's email: sdoyle.email@gmail.com

Additive manufacturing techniques have become increasingly important for fabricating 3D tissue engineering architectures since their introduction to the biomaterials field at the turn of the millennium [1]. While the last two decades have seen remarkable advances in the application of 3D printed biomaterials [2], current fabrication methods are still limited by: (i) a relatively poor resolution, (minimum size of structural elements is typically 200-1000µm); (ii) poor versatility of materials (new materials typically need to be laboriously optimised for printing); (iii) an inability to create highly porous (>80% empty space) structures, necessary for many biological applications; (iv) limitations in structural complexity, particularly in generating interconnected pores facilitating anisotropic diffusion.

Here, we present a new method of fabrication in which we overcome each of these limitations for a wide range of materials including biodegradable polymers, resins, low-melt metal alloys, silicones, ceramics and hydrogels. Our strategy uses a novel templating approach inspired by the ancient metallurgical technique of 'lost-wax' or 'investment' casting which has been used for making complex sculptures for at least 6,000 years [3]. Our high degree of control over feature size and design complexity enables the create of scaffolds over a range of porosities (from 50% to 97%) which translates to compressive stiffness's of over three orders of magnitude (from 10 kPa to >10,000 kPa). This provides the technical platform for creating tissue engineering scaffolds relevant to a range of body tissues, from breast tissue to an array of cartilages.

Keywords

Universal fabrication; Material versatility ; Design complexity

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Abstract **#248**

ENGINEERING THE COMPLEX MICRO-ENVIRONMENT OF THE BLOOD-RETINAL BARRIER IN VITRO

Ashley R Murphy$_P$[1], Grace Lidgerwood[2,3], Alice Pébay[2,3], Yen B Truong[1], Carmel M O'Brien[1,4], Veronica Glattauer[1]

[1] *CSIRO Manufacturing, Research Way, Clayton, Australia*

[2] *Department of Anatomy and Neurosciences, The University of Melbourne, Parkville, Australia*

[3] *Department of Surgery, Royal Melbourne Hospital, The University of Melbourne, Parkville Australia*

[4] *Australian Regenerative Medicine Institute, Monash University, Innovation Walk, Clayton, Australia*

Corresponding author's email: Ash.Murphy@csiro.au

Retinal degenerative disorders such as age-related macular degeneration (AMD) are a leading cause of vision loss worldwide (1). Medications can slow the progression of some forms of AMD, however, there are currently no treatments to halt or reverse their progression (2). In vitro retinal models present as a high-throughput and cost effective option for studying retinal disorders, however current lack of complexity inhibits their potential to translate outcomes clinically (3-5). A fibrous, acellular layer known as Bruch's membrane, supports the retinal pigment epithelium (RPE) and maintains separation of the retina from the underlying choroidal vasculature. Interactions between RPE and Bruch's membrane are critical in the pathogenesis of AMD.

Commonly used substrates for the culture of RPE cells in vitro, such as synthetic Transwell® membranes, fail to replicate the biomaterial properties of the native human Bruch's membrane (6). We describe here the fabrication and characterisation of ultrathin functionalised collagen membranes that more accurately mimic the native properties of Bruch's membrane. Here we present data elucidating material physical and biochemical properties. We also present material biocompatibility outcomes with the human RPE cell line, ARPE-19. Finally, we highlight the design and fabrication of a membrane perfusion system that allows for robust handling of ultrathin membrane cultures and provides media flow to mimic that of the choroid and retinal vasculature. Together with human pluripotent stem cell-derived RPE cells this system aims to accurately mimic the in vivo microenvironment of the RPE, Bruch's membrane and choroid to a give greater insight into AMD disease mechanisms.

Keywords

retina; membrane; perfusion

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Abstract **#249**

PRECISE ORCHESTRATION OF GROWTH FACTORS FOR FUNCTIONALIZATION OF CHONDROACTIVE SCAFFOLDS TOWARDS SELF-DIFFERENTIATING HUMAN-ADIPOSE DERIVED STEM CELLS FOR CARTILAGE REPAIR

Lilith Caballero Aguilar$_P$[1,2], Serena Duchi[1,3], Carmine Onofrillo[1,3], Cathal O'Connell[1,4], Claudia Di Bella[5,3]

[1] *BioFab3D, St. Vincent's Hospital Melbourne*

[2] *Swinburne University of Technology*

[3] *Department of Surgery, University of Melbourne*

[4] *Faculty of Engineering, RMIT*

[5] *St. Vincent's Hospital Melbourne*

Corresponding author's email: lcaballeroaguilar@swin.edu.au

Formation of building-tissue in a uniform manner within a matrix is a major challenge in tissue engineering. This problem arises for various reasons. Prevalently, the lack of nutrients at the core of scaffolds hinders the ability of stem cells to develop tissue homogeneously across the scaffold (1-2). For cartilage formation, the use of human adipose-derived stem cells (hADSCs) and their differentiation towards chondrogenic lineage by BMP-6 and TGFb-3 growth factors have been proposed (3). However, the precise amount and rate at which hADSCs consume and demand these growth factors within cell-laden scaffolds still largely unknown (2). To address this, we first performed a detailed investigation of the minimal amount, dosages, and single-factor dependence that hADSCs required to trigger differentiation. The elucidation of the minimal dosage then led us to investigate the fabrication of a chondroactive scaffold using gelatin methacryloyl (GelMa) functionalized with a thiol-modified-heparin to sequentially deliver BMP-6 and TGFβ-3. This strategy against the bolus integration and periodic exogenous dosage of growth factors was investigated by metabolic assays, mechanical compression, swelling ratio tests and photorheological measurements. The scaffolds with controlled sequential release triggered production of Collagen type I and type II across the scaffold. In contrast in the exogeneous delivery group, mostly peripheral matrix accumulation was observed. Orchestrating the delivery by controlling growth factors rate resulted in higher matrix accumulation when compared to the growth factors alone within the scaffold. These results introduce a step forward in the creation of self-chondrogenic constructs for cartilage repair.

Keywords

Growth factors; Heparin; GelMa

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Abstract **#252**

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FOR THE DISEASE MODELLING OF DIGEORGE SYNDROME

Dorian Luijkx[P][1,2]

[1] *MERLN Institute for Technology-inspired Regenerative Medicine*

[2] *RIKEN BioResource Research Center*

Corresponding author's email: d.luijkx@maastrichtuniversity.nl

DiGeorge Syndrome is a disorder caused by microdeletions in chromosome 22q11.2, and causes symptoms such as congenital heart defects, poor immune system, and/or hypoparathyroidism. The mechanisms behind the developmental defects that shape its phenotype are still largely unknown. One of the biggest hurdles is that, despite the consistent size of the deletion, the symptoms of the syndrome are widely variable and the expression and penetrance of the symptoms show a high level of heterogeneity between patients (1). For these reasons, cells obtained from a high number of unrelated patients are required to study the development of DiGeorge Syndrome in a meaningful way. This disorder affects the development of pharyngeal arches during early embryogenesis, therefore stem cells or progenitors derived from patients would be the ideal cell resource for examining the flaws in the developmental programs of various affected lineages (2, 3). However, the use of primary stem cells is ethically and practically complicated. Induced pluripotent stem cells offer a solution since they can be generated from differentiated, readily available cells, such as fibroblasts (4, 5).
We here report the episomal vector-mediated reprogramming of three commercially available fibroblast lines derived from DiGeorge Syndrome patients to iPSCs. These cells were characterized based on their expression of established pluripotency markers and their ability to differentiate into derivatives of all three germ layers in vitro. After more extensive characterization, these cell lines can be applied to study the development of DiGeorge Syndrome in vitro, paving the way towards the development of new therapies.

Keywords

DiGeorge Syndrome; generation; disease model

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Abstract **#253**

FABRICATION OF PANCREATIC SPHEROIDS WITH NEURON AND VASCULAR NETWORKS

Dina Myasnikova$_P$[1], Shoji Takeuchi[1]

[1] *The University of Tokyo*

Corresponding author's email: r-hybrid-dina@g.ecc.u-tokyo.ac.jp

Fabrication of bioartificial islets in the form of pancreatic spheroids (spherical multicellular aggregates) with further transplantation is one of the emerging and highly promising regenerative medicine approaches for diabetes treatment. However, its more active development and application are hampered by the lack of vascular structure inside spheroids. The latter leads to a high percentage of posttransplantation apoptosis due to hypoxia in the core of spheroids and limits the transplantable spheroid size to approximately 100 μm.

The later research shows that apart from the vascular network, the presence of neurons during embryonic development is essential for the formation of the correct architecture of pancreatic islets and their postpartum function. Moreover, some research shows that vascular and neuron cells tend to promote the formation of their networks mutually. Thus, in our work, we investigate the possibility to facilitate vascular network formation by using co-culture of three types of cells: pancreatic beta-cells, vascular endothelial and neuron stem cells. As the process of network formation takes several weeks, we used polydimethylsiloxane microwell array device for spheroid fabrication, which allows decreasing hypoxia in the spheroid core and thus enables long-term culture. Our preliminary results showed the formation of neuron network and elongation of vascular cells. Although more optimization is needed to perfect this approach, we believe that it would be valuable for the investigation of neuron and vascular cells interactions inside pancreatic islets and their influence on islet functioning.

Abstract **#254**

3D PRINTING PARAMETERS CORRELATED WITH THE THIXOTROPIC BEHAVIOR OF GELATIN METHACRYLOYL

Fernanda Condi de Godoi$_P$[1], Kristof Flebus[2], Bart Thewissen[1], Chris Peetermans[1]

[1] *Tessenderlo Innovation Center*

[2] *KU Leuven*

Corresponding author's email: fernanda.godoi@tessenderlo.com

This study correlates the thixotropic behaviour of gelatin methacryloyl (GelMA) with its printing parameters in extrusion printing. GelMA provided by Claro (BGI800) was compared with a conventional GelMA. The experiments were conducted without photoinitiators and constant GelMA concentration (10%w/w) using an Inkredible+ printer at 21 ± 1°C. Parameters like expansion degree, broadening degree and filament spreading were determined for one-dimensional printing. The aspect ratio of 3D printed lattices was calculated by the ratio between printed areas and designed areas (Pr). The thixotropic behaviour was evaluated by a three-interval oscillatory-rotational-oscillatory (ORO) which simulates, respectively: (1) “in the cartridge” condition, (2) flow during printing at 1000s-1 (30 seconds) and (3) recovery of the filament after extrusion. Our study revealed that stable and continuous printing behaviour is achieved by conducting the experiments slightly above the gelation temperature (Tgel) of GelMA. Thus, BGI800 with Tgel = 20°C demonstrated an advantage over the conventional GelMA with a Tgel = 24°C. The lower storage modulus (G’) at rest (ORO test) is associated with lower printing pressures (45-70kPa for BGI800 against 200-304kPa for conventional GelMA). Fast recovery after shear can be assigned to shape-fidelity, however, the extremely fast recovery observed for conventional GelMA at 21°C led to the formation of a brittle gel (high G’), resulting in low Pr and a high expansion degree. Our findings demonstrate that thixotropy is effectively correlated with printing parameters. This approach is a powerful tool to objectively benchmark and to compare bioinks, and aids in the design of improved printing behaviour.

Keywords

GelMA; thixotropy; printability

Abstract **#255**

RESCUE INFLAMMATION DESTROYED TENOGENIC DIFFERENTIATION BY INHIBITION OF NF-KB PATHWAY

Jialin Chen[P][1], Ludvig J. Backman[2], Wei Zhang[1]

[1] *School of Medicine, Southeast University, Nanjing, China*

[2] *Department of Integrative Medical Biology, Anatomy, Umeå University, Umeå, Sweden*

Corresponding author's email: kiven99999@163.com

Inflammation after injury influence the repair effect of tendon. However, its exact roles and inherent mechanism in tenogenic differentiation of tendon stem cells are unknown. This study evaluated the regulation effect on tenogenic differentiation of two main inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). It is found that both IL-1β and TNF-α inhibited tenogenic differentiation by decreasing the expression of tendon markers and increasing the expression of MMPs. NF-κB pathway was found to be involved during this process. Treatment by TPCA-1, one inhibitor of NF-κB pathway, rescued the inflammatory cytokines destroyed tenogenic differentiation potential of tendon stem cells. Furthermore, sustained release of TPCA-1 from silk scaffold promoted the tendon repair and regeneration. These results collectively show the roles and mechanism of inflammation in tenogenic differentiation. It is feasible to improve tendon repair and regeneration by modulating the inflammation related pathways such as NF-κB pathway.

Abstract **#258**

TOWARDS THE BIOHYBRID LUNG: COMPUTATIONAL FLUID DYNAMICS ASSESSMENT OF HOLLOW-FIBER MEMBRANE OXYGENATORS

Daniele Dipresa[1], Michael Pflaum[1], Ariana Monica Silva Peredo[1], Axel Haverich[1], Sotirios Korossis[2]

[1] Lower Saxony Centre for Biomedical Engineering Implant Research and Development, Hannover Medical School, Hannover, Germany; Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany

[2] Lower Saxony Centre for Biomedical Engineering Implant Research and Development, Hannover Medical School, Hannover, Germany; Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany; Cardiopulmonary Regenerative Engineering (CARE) Group, Centre for Biological Engineering, Loughborough University, United Kingdom

Corresponding author's email: dipresa.daniele@mh-hannover.de

Extracorporeal membrane oxygenation (ECMO) is the gold standard for treating respiratory failure, as a bridge to lung transplantation. However, ECMO devices have several issues (max usage to 4 weeks). Thrombus formation is a cause of failure, which limits gas-transport and is associated to device haemodynamics. Endothelialisation of the hollow-fibres (HF) membrane offers a potential solution to thrombogenicity. However, inhomogeneous haemodynamics would subject the seeded endothelial cells (ECs) to variable and potentially damaging blood wall shear stress (WSS).

CFD models of a paediatric (MiniLung) and an experimental (RAT) oxygenator were developed to mimic flows up to 0.8 l/min with blood, DMEM (for EC seeding) and water (for CFD validation). CFD models predicted pO2, O2 saturation, velocity and WSS under different HF configurations on the outer HF walls.

The presence of inhomogeneous WSS on the HF oxygenators was observed among the three fluids in both models. The blood-based models predicted higher WSS compared to the DMEM- and water-based models. Lower blood velocity was associated with low WSS (<0.1 Pa) and areas of recirculating flow. A higher incidence of thrombus formation and clotting is expected to occur in such regions. The results suggested that O2 diffusion resistance across the HF wall and fibers configuration played a role in O2 transport efficiency and WSS distribution.

The CFD models predicted regions of variable WSS, including stagnant and recirculating flow regions, suggesting increased flow-induced blood damage and clotting formation. The predictions highlighted the need for design optimisation in order to minimising thrombus formation allowing efficient endothelialisation.

Abstract **#259**

INSPIRED BY DEVELOPMENT: WNT-ENHANCED MESODERM COMMITMENT BOOSTS CARTILAGE TISSUE YIELD FROM INDUCED PLURIPOTENT STEM CELLS

Solvig Diederichs[P][1], Ursula Kreuser[1], Wiltrud Richter[1]
[1] *Research Center for Experimental Orthopaedics, Heidelberg University Hospital*
Corresponding author's email: solvig.diederichs@med.uni-heidelberg.de

Induced pluripotent stem cell (iPSC)-derived chondrocytes are currently the only cells that match the ability of primary articular chondrocytes to form cartilage in vitro without succumbing to hypertrophic degeneration. However, in vitro differentiation of iPSCs first into mesoderm and then into chondrocytes is currently insufficiently stringent and cell selection procedures are applied to dispose of misdifferentiated cells, thus causing high cell loss and low tissue yield. During embryonic development a WNT/β-catenin pulse initiates mesoderm commitment. We here hypothesized that an initial WNT/β-catenin pulse could enhance mesoderm commitment of iPSCs and thereby overcome limited cartilage yield.

In two human iPSC lines a short initial WNT/β-catenin pulse with CHIR99021 enhanced mesodermal marker expression, supported exit from pluripotency and inhibited ectodermal misdifferentiation, thus indicating enhanced mesodermal commitment. Importantly, the initial CHIR pulse increased expression of multiple extracellular matrix (ECM)-related genes, adjusted adhesion-related gene expression (CDH3 up, CDH6 down), and yielded more matrix-interacting progenitors with high aggregation capacity. Subsequently, the majority of CHIR-induced cells contributed to chondrogenic pellet formation, thus increasing cell yield after eight weeks 200-fold compared to controls. Moreover, an enlarged collagen-II and proteoglycan-positive area in the CHIR group indicated an increased number of cartilage-forming cells.

Consequently, short initial WNT-activation acted via improved mesoderm commitment, ECM expression and cell aggregation and made cell selection steps before chondrogenesis obsolete. This robust and efficient iPSC differentiation into high-quality mesodermal progenitors rescued low cartilage yield during subsequent chondrogenesis, which is a major step towards iPSC application in clinical cartilage regeneration, disease modelling and drug screening.

Keywords
induced pluripotent stem cells; WNT; development

Abstract **#260**

A COMPARATIVE STUDY OF COMPUTATIONAL MODELLING OF YAP/TAZ NUCLEAR TRANSLOCATION IN 2D AND 3D

Kerbaï Saïd Eroumé[1], Kateřina Staňková[2], Rachel Cavill[2], Jan de Boer[3], Aurélie Carlier[1]

[1] *MERLN Institute, Maastricht University, Maastricht, the Netherlands*

[2] *Department of Data Science and Knowledge Engineering, Faculty of Science and Engineering, Maastricht University, Maastricht, the Netherlands*

[3] *Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, the Netherlands*

Corresponding author's email: k.eroume@maastrichtuniversity.nl

Introduction

YAP/TAZ, a transcriptional co-activator, has been shown to play an important role in mechanosensing, although the mechanism is unelucidated [1].

Here, we explore the effect of cell dimension on YAP/TAZ nuclear translocation, by extending the 1D mechanosensing model in [2] to 2D and 3D reaction-diffusion equivalents, which we implemented in VCell [3].

Methods

Parameter values and diffusion coefficients are according to [2,4] and the Stokes-Einstein equation [5] respectively. Model predictions were tested on 2D square cells (44.6µm x 44.6µm) and 3D cuboidal cells (15µm x 15µm x 26µm). The FAK activation occurred in a ring close to the cell membrane in 2D, or one side of the cube in 3D.

Results

For similar FAK activation areas and FAK to nucleus perpendicular distances, the temporal evolution of the concentration of molecules was faster in 2D than in 3D. The peak concentrations were attained more gradually in 3D, with active RhoA having a higher peak concentration in 3D. The YAP/TAZ nuclear fraction was higher in 3D than in 2D.

Discussion

Our preliminary results reveal that the difference in YAP/TAZ nuclear translocation in 2D compared to 3D are opposite to the experimental results of Caliari et al. [6]. Future work will focus on extensively investigating the effect of cell size and shape in 2D and 3D to further understand YAP/TAZ nuclear translocation.

Acknowledgements

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Keywords

Computational modelling; YAP/TAZ; Nuclear translocation

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Abstract **#261**

DEVELOPMENT OF A VASCULARISED BONE MODEL TO STUDY ROLE OF VASCULATURE ON OSTEOARTHRITIS ONSET

Alessandro Pirosa[P][1], Andrea Mainardi[1,2], Paola Occhetta[2], Martin Erhbar[3], Ivan Martin[1], Marco Rasponi[2], Andrea Barbero[1]

[1] *University Hospital Basel, Basel, Switzerland*

[2] *Politecnico di Milano, Milano, Italy*

[3] *University Hospital Zurich, Zurich, Switzerland*

Corresponding author's email: alessandro.pirosa@usb.ch

The main clinical indicators of osteoarthritis (OA) include loss of articular cartilage, altered subchondral bone remodeling/vascularization, and inflammation. Given the complexity of OA, multi-cellular and complex human in vitro models are needed to tackle different aspects of the pathology. We developed an engineered vascularized bone (VB) tissue using a mesenchymal stromal cell line (MSOD) and HUVEC within photocrosslinkable gelatin methacrylate as 3D matrix. After verifying MSOD osteogenesis and HUVEC tubulogenesis during an initial culture phase of 2 weeks, we challenged our model with an inflammatory cocktail (IL-6, IL-1β and TNFα) for an additional week, to assess whether OA features could be recapitulated. Inflammation caused demineralization and increased ALP activity, especially in the presence of HUVEC, confirming the role of vasculature in the development of OA. RT-PCR analyses demonstrated an increased VEGF and decreased Wnt5a expression in the MSOD-HUVEC group compared to MSOD control. We also found higher TGFβ-1 in HUVECs upon inflammation (TGFβ-1 is an autocrine proangiogenic signal in response to inflammation). An additional OA feature we were able to model was the abnormal collagen type 1 (Col1) production, showing increased Col1α1/Col1α2 chain ratio in MSOD-HUVEC in the presence of inflammation, which might explain demineralization due to limited capacity of this form of collagen of retaining hydroxyapatite crystals. After verifying the generation of such OA phenotypes, we will integrate VB with a cartilage layer within a microfluidics platform and trigger OA by inflammatory and mechanical stimulation, to identify molecular pathways that can be targeted to reverse the disease.

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Abstract **#262**

BIOENGINEERING THE CORNEAL ENDOTHELIUM: A CORNEAL ENDOTHELIAL GRAFT USING BIOMIMETIC SUBSTRATES

Pere Català P[1,2,3], Oleksandr Zagorodko[4,3], Annika F. Vrehen[4,3], Rudy M.M.A. Nuijts[2,3], Patricia Y.W. Dankers[4,3], Mor M. Dickman[2,3], Vanessa L.S. LaPointe[1,3]

[1] Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

[2] University Eye Clinic Maastricht, Maastricht University Medical Center+, Maastricht, the Netherlands

[3] EyeSciTe, Chemelot-InSciTe, Maastricht, the Netherlands

[4] Department of Biomedical Engineering, Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, the Netherlands

Corresponding author's email: p.catala@maastrichtuniversity.nl

The cornea is the clear window that lets light into the eye. The inner surface of the cornea is lined by corneal endothelial cells (CECs) that function as an active metabolic pump to maintain the transparency of this avascular tissue. Corneal disease is one of the leading causes of blindness and visual disability, affecting over 15 million people worldwide. In developed countries, corneal endothelial disease is responsible for most corneal opacities. State of the art therapy involves selective replacement of the corneal endothelium with that of a donor. However, only one donor cornea is available for every seventy patients in need.

Endothelial cell transplantation could reduce the dependence on cadaveric donor tissue. In this project, we aim to produce CECs for transplantation and engineer corneal endothelial grafts in order to effectively transplant the CECs.

Human artificial corneal endothelial grafts were developed by culturing primary human CECs on biomimetic material electrospun meshes. The grafts achieved a high CEC density (the primary quality criterion for regular donor corneas) while maintaining the transparency of the construct. Moreover, the grafts showed the characteristic endothelial cell makers: zona occludens-1 (ZO-1), and CD166. The detection of Na+/K+ ATPase suggested proper cellular pump function was intact in the construct.

Overall, we have successfully developed biomimetic electrospun meshes with biological properties that supported the culture and phenotype maintenance of primary human CECs. The resulting artificial corneal endothelial construct offers potential utility for development of implantable tissue-engineered cell-carrier corneal endothelial constructs.

Keywords

Corneal endothelium; Human primary culture; Tissue-engineered cell carrier

Abstract **#263**

AN EX VIVO OSTEOCHONDRAL CULTURE PLATFORM FOR BONE REMODELLING RESEARCH

Esther Cramer$_P$[1], Nicole van Gestel[1], Keita Ito[1], Sandra Hofmann[1]

[1] *Orthopaedic Biomechanics Group, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, the Netherlands*

Corresponding author's email: e.e.a.cramer@tue.nl

The preclinical testing process for novel therapies for damaged and diseased bone usually consists of in vitro cell experiments followed by in vivo animal studies. The correlation between the outcomes of these in vitro and in vivo studies is poor, which hampers translation into clinical practice [1]. Ex vivo explant cultures could provide a closer representation of the in vivo situation because they maintain all bone cells in their native 3D environment. The aim of this research is to establish an ex vivo culture platform to study bone remodelling. The platform is based on an explant culture model for cartilage defects [2,3].

Osteochondral cores were isolated from porcine femoral condyles and cultured for 6 weeks. Cell metabolic activity was monitored, and histological analysis was performed. Osteoclast activity was measured with TRAP assay and µCT was performed weekly to quantify changes in the amount of mineralized tissue to acquire insight into bone remodelling over time.

Cell metabolic activity was preserved for the tested culture period and cell nuclei were still present in marrow tissue as well as in trabeculae after culture. TRAP activity in the medium indicated the presence of active osteoclasts over the first 9 days only, whereas µCT imaging demonstrated an increase in mineralized volume (9.1 ± 1.4%) and surface (4.2 ± 2.4%) after 6 weeks of culture.

The ability to preserve cell viability and the opportunity to monitor bone formation and -if present- resorption shows a first step towards development of a testing platform to study bone remodelling.

Keywords

Bone remodelling; Osteochondral explant; Porcine

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Abstract **#264**

HUMAN FETAL LUNG MESENCHYMAL STEM CELLS: CHARACTERIZATIONS AND POTENTIAL FOR CELL THERAPY OF ACUTE LUNG INJURY AND FIBROSIS

Mahtab Golmohammadi[1,2], Fatemeh Ganji[1,3], Ali shirani[1,3], Ali Samadikuchaksaraei[1,3,4]

[1] *Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran*

[2] *Department of Genetic, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

[3] *Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran*

[4] *Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran*

Corresponding author's email: golmohammadimahtab@gmail.com

Objective: Despite decades of research, no specific pharmacological therapy to treat acute lung injury (ALI) has been identified. Mesenchymal stem cells (MSCs) are one attractive therapeutic approach. In this study, we aim to investigate the effects of human fetal lung mesenchymal stem cells (hFL-MSCs) in an animal model of bleomycin-induced ALI and fibrosis.

Methods: hFL-MSCs were isolated from lung tissues derived from spontaneously aborted fetuses at 16 and 18 weeks of gestation. After characterization of MSCs, the model animals received intratracheal hFL-MSCs after bleomycin instillation. To assess the effect of cell therapy, all experimental groups were analyzed for cell count and level of cytokines in bronchoalveolar lavage fluid (BALF) at days 3, 7, 14, and 28 post-cell administration. After lung tissue harvest, all tissue changes were evaluated by immunostaining and wet/dry weight ratio and followed by an examination of expression of inflammatory and anti-inflammatory genes.

Results: FL-MSCs highly expressed CD105, CD90 and CD73, did not revealed expression of CD45, CD34 and HLA-DR and showed the capacity for trilineage differentiation. Histopathological analyses in hFL-MSCs therapy groups showed a decrease in infiltration of inflammatory cells and wet/dry weight ratio in lung tissues in compared to untreated group. Also, ELISA test was demonstrated the decrease in TNF-? and IL-6 in BALF. The levels of fibrosis at each time point were significantly reduced by hFL-MSCs treatment.

Conclusion: These data revealed a decrease in inflammatory cytokines and progression of fibrosis following hFL-MSCs administration.

Keywords: fetal lung; mesenchymal stem cells; bleomycin; acute lung injury; fibrosis

Abstract **#265**

LABEL-FREE RAMAN MICROSCOPY-BASED MONITORING OF ANTI-CANCER DRUG EFFECTS IN COLORECTAL CANCER ORGANOIDS TOWARDS PATIENT-INDIVIDUALIZED OPTIMIZATION OF TREATMENTS.

Lucas Becker[P][1,2], Julia Marzi[1,2,3], Daniel Carvajal Berrio[4,2], Thomas E Mürdter[5], Phillip Renner[5,6], Marc H Dahlke[5,6], Anne T Nies[2,5], Katja Schenke-Layland[4,7,3], Matthias Schwab[5,8,9], Nicole Janssen[6]

[1] *Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany*

[2] *Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", University of Tübingen, Germany*

[3] *Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany*

[4] *Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany*

[5] *Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology and University of Tübingen, Stuttgart, Germany*

[6] *Department of Surgery, Robert Bosch Hospital, Stuttgart, Germany*

[7] *Department of Medicine / Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA*

[8] *Department of Biochemistry and Pharmacy, University of Tübingen, Tübingen, Germany*

[9] *Department of Clinical Pharmacology, University Hospital, Tübingen, Germany*

Corresponding author's email: lucas.becker@uni-tuebingen.de

Primary and acquired therapy resistance of solid tumors are major problems in clinical oncology including different mechanisms such as decreased drug influx and/or increased drug efflux. To study and understand variability of such effects novel drug-screening models are required. Of particular interest are primary patient-derived tumor-organoids recapitulating in vivo tumor-biology, since they represent the interaction of cellular and extracellular environments. Of note, expression of genes encoding to enzymes involved in adsorption, metabolism and excretion of drugs are preserved in such culture systems. Here, we describe non-invasive imaging procedures (i.e. Raman-microscopy, Fluorescence Lifetime Imaging Microscopy (FLIM)) allowing label-free and real-time cellular analysis and monitoring effects of drug-treatment. A biobank of colorectal cancer (CRC) organoids was established from surgical specimens and cultivated in Matrigel. Cellular response of organoids upon drug-treatment was analyzed in real-time by Raman-microscopy after applying CRC relevant cytotoxic agents. Spectral fingerprints can be used to identify and image chemical compositions in living biological specimens. To assess information about metabolic activity FLIM was used to monitor the local fluorophore environment of NADH and FAD. Through multivariate data analysis Raman-spectra of treated and non-treated tumor-organoids were compared showing the effects of drugs on e.g. nuclei, cytoplasm and mitochondria by shifts of specific molecular vibrations.Overall, Raman-imaging is an enabling technology to evaluate tumor-drug interactions in a non-invasive manner, with the possibility to screen for patient-individualized effectivity optimizing drug-treatment.Supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy - EXC 2180-390900677 and the Robert Bosch Stiftung, Stuttgart, Germany.

Abstract **#266**

KEY REGULATOR OF PANCREATIC DEVELOPMENT PDX1 INCREASES CELL ADHESION AND REDUCES METASTATIC POTENTIAL OF PANCREATIC CANCER CELLS

Liya Kondratyeva[P][1], Igor Chernov[1], Eugene Sverdlov[2]

[1] *Shemyakin-Ovchinnikov Institute of bioorganic chemistry of Russian Academy of Science*

[2] *Institute of Molecular Genetics of National Research Centre «Kurchatov Institute»*

Corresponding author's email: liakondratyeva@yandex.ru

Metastases are the main cause of death of pancreatic ductal adenocarcinoma (PDAC) patients and often occur at early stages of PDAC development. In cancer biology, metastasizing is one of the most poorly studied phenomena. Recently, the attention of researchers was attracted by PDX1, the key regulator of the pancreas development, as possible factor involving in suppression of spreading cancer cells. This work is devoted to the study of the role of the PDX1 gene in the progression of PDAC.

PDX1 expression is absent in 6 model PDAC cell lines. To study the effect of PDX1 overexpression on the malignant potential of pancreatic cancer cells, we transduced PANC-1 and Colo357 cells by PDX1-carrying lentivirus. It was shown that PDX1-positive PANC-1 and Colo357 cells possess increased growth rate of 1.7 ($p < 0.01$) and 1.6 times ($p < 0.05$), respectively. Migration through Transwell and the rate of wound healing in PANC-1 and Colo357 cells expressing PDX1 were reduced by 50% compared to control cells ($p < 0.01$). Collagen I and Fibronectin adhesion assay was shown increased adhesion rate PDX1-positive PANC-1 and Colo357 cells relative control cells up to 2 fold. Analysis of motility related genes expression revealed changes in genes of regulators epithelial-to-mesenchymal transition, extracellular matrix proteins, cell-to-cell contacts and growth factors. Our results may indicate that expression of the PDX1 gene inhibits metastatic potential of pancreatic cancer cells via increasing of cell adhesion.

The study was supported by the Russian Science Foundation (project no.14-50-00131).

Abstract **#267**

MECHASCAN: A NOVEL ONLINE MONITORING TOOL FOR ASSESSING MECHANICAL PROPERTIES OF TISSUE ENGINEERED GRAFTS

Lu Luo[P][1], Jonathan Mason[2], Matteo Taffetani[3], Sarah Waters[3], Yvonne Reinwald[4], Ying Yang[5], Pierre Bagnaninchi[2], Alicia El Haj[1]

[1] *University of Birmingham*

[2] *University of Edinburgh*

[3] *University of Oxford*

[4] *Nottingham Trent University*

[5] *Keele University*

Corresponding author's email: l.luo.2@bham.ac.uk

Tissue engineering strategies provide a promising approach for replacing diseased and damaged tissues in patients. Monitoring mechanical properties of engineered grafts during culture and characterising them prior to implantation into patients is important for defining their ultimate quality and performance. However, most traditional mechanical testing approaches are non-sterile and destructive. We present here a novel technique which utilizes new approaches for using Optical Coherence Tomography (OCT) for non-invasive online monitoring mechanical properties of engineered tissues. We first validated this technique using acellular tissue phantoms. 1%, 2% and 3% agarose gels were fabricated and imaged using OCT. The Young's modulus of the sample was computed by novel algorithms identifying key parameters in the OCT images. The computed Young's moduli correlated well with those obtained by traditional mechanical testing approach. Next, we validated it using engineered bone tissues. Mesenchymal stem cells (MSCs) were pelleted and cultured in an osteogenic differentiation medium for 3 weeks. Samples were continuously monitored in a contactless and sterile manner on day 3, 10 and 21, and meanwhile some samples were harvested for mechanical testing on the same time points. The Young's modulus of the cell pellet was computed using MECHASCAN. As tissues matured in culture, MECHASCAN analysis showed a significant increase in the computed Young's moduli, correlating with the increase in those values obtained by traditional mechanical testing approach. In conclusion, we demonstrated here a new OCT based platform for sterile online monitoring of mechanical properties of tissue engineered grafts.

Abstract **#268**

A 3D EXTRACELLULAR MATRIX HYDROGEL DERIVED FROM THE LEFT VENTRICLE OF PORCINE MYOCARDIUM SUPPORTS VASCULAR NETWORK FORMATION

V. E. Getovaᴘ[1], Gabriel R. Liguori[1,2], Luiz Felipe P. Moreira[2], Janette K. Burgess[1], Martin C. Harmsen[1]

[1] *University of Groningen, Medical Center Groningen, Department of Pathology and Medical Biology, Groningen, Netherlands*

[2] *Instituto do Coração (InCor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil*

Corresponding author's email: getova.vasilena@gmail.com

INTRODUCTION: After myocardial infarction, restoration of local perfusion of the damaged myocardium is essential. Perfusion depends on a branched vascular network which aids tissue repair while also suppressing extensive fibrosis. Vascular network formation is enhanced by extracellular matrix (ECM). Preliminary evidence showed that myocardial-derived ECM hydrogels supported pericyte-driven vascularization by microvascular endothelial cells (HPMEC) [1]. In the current study, we assessed the conditions (seeding density, culture time, type of endothelial cells (EC)) and the mechanism that drives pericyte-free vascularization of myocardial hydrogels.

METHODS: Left ventricles (LV) of porcine hearts were decellularized, freeze-dried and pepsin-digested to prepare pre-gels. Pre-gels were mixed with microvascular ECs (HPMEC) or macrovascular EC (HUVEC) in different cell densities (9^5 to 10^3 EC/ml). Cultures were monitored for >30 days and network formation assessed by fluorescent 2D and 3D microscopy.

RESULTS: The LV ECM hydrogel supported vascular network formation of microvascular endothelial cells in 3D. The seeding density of 10^3 EC/ml was optimal, network formation started at day 2 and was stable for more than 30 days. Also, the ECM hydrogels supported vascular network formation of macrovascular endothelial cells with pericytes in 3D, in a ratio 1:1 (in total 10^3 EC/ml).

DISCUSSION & CONCLUSIONS: Left ventricular porcine ECM hydrogels support vascular network formation by microvascular and macrovascular ECs in 3D. This work can lead to the development of a tissue-engineered implant to stimulate neovascularization and tissue regeneration.

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Abstract **#269**

FROM THE LAB INTO THE CLINIC: ELECTROSPINNING OF MEDICAL DEVICES

Mehri Behbehani$_P$[1], Matei Cirstea[1], Ann Kramer[1], Rob McKean[1]

[1] *The Electrospinning Company*

Corresponding author's email: mehri.behbehani@electrospinning.co.uk

Electrospinning has received an ever-increasing amount of attention academically, as judged by the rise in the number of publications over the last three decades. It has been established as a fast and versatile fabrication technique for fibrous materials for use in implantable biomedical devices. Nevertheless, there is significant discrepancy between the academic effort undertaken so far and the relatively low number of approved medical devices utilising electrospun materials. The question therefore remains why this scientific knowledge can rarely be translated into the clinic.

Over the years, The Electrospinning Company has developed to a centre of excellence in biomedical electrospinning and we have a thorough understanding on how to achieve biomaterial characteristics such as mechanical strength, resorption time and architecture, suitable for specific therapeutic indications and target tissues. Having worked with many academic research groups as well as industrial clients, we have seen the challenges involved in moving scientific research to the market. We currently fabricate biomaterials that are used as part of an FDA-approved orthopaedic medical device, and others that are being evaluated in clinical trials in different indications and regions.

In this presentation we will provide an overview of the status of electrospun biomaterials on, or close to, market and share the challenges associated with taking promising electrospinning research out of the lab and into the clinic. We will further focus on how we approach device design and manufacture in order to maximise the chances of clinical and commercial success, and will illustrate this with a case study.

Abstract **#270**

GUIDING CARTILAGE REGENERATION USING LOCAL PROTEIN IMMOBILISATION IN MELT ELECTROWRITTEN MICROFIBRE SCAFFOLDS

Madison Ainsworth[P1,2], Oliver Lotz[3,4,5], David McKenzie[5], Marcela M.M. Bilek[3,4,5], Jos Malda[1,2,6], Behnam Akhavan[3,4,5], Miguel Castilho[1,2,7]

[1] Regenerative Medicine Centre Utrecht, University Medical Center Utrecht, Utrecht, the Netherlands

[2] Department of Orthopaedics, University Medical Center Utrecht, Utrecht, the Netherlands

[3] School of Biomedical Engineering, University of Sydney, NSW, Australia

[4] School of Aerospace, Mechanical & Mechatronic Engineering, The University of Sydney, NSW, Australia

[5] School of Physics, University of Sydney, NSW, Australia

[6] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

[7] Department of Biomedical Engineering, Technical University of Eindhoven, Eindhoven, the Netherlands

Corresponding author's email: M.J.Ainsworth-2@umcutrecht.nl

There is no mechanically competent, biologically functional treatment for end-stage cartilage degeneration. In this study, we hypothesise that the fabrication of well-organized microfibre reinforcing scaffolds[1] with locally, covalently immobilised TGFβ1 could support and guide the formation of new cartilaginous tissue. To create a complex mechanical structure with the necessary biomedical cues[2], we combined melt electrowriting (MEW) and atmospheric-pressure plasma (APP) to produce well-organized microfibre scaffolds with selectively, covalently-immobilized TGFβ1.

Poly-e-caprolactone MEW scaffolds were fabricated using a 3DDiscovery (RegenHU), then functionalised using a computer-controlled APP device, generating a controlled functionalisation pattern. TGFβ1 was then immobilized onto the MEW scaffold and covalent attachment was validated using FTIR spectroscopy and immunofluorescence detection. In vitro experiments were performed by seeding equine MSCs into MEW scaffolds and were cultured for 28 days. Neo-cartilage formation was quantified for glycosaminoglycan (GAG) production and confirmed with histological analysis.

Covalent immobilisation of TGFβ1 was achieved using the APP-functionalisation approach. FTIR confirmed a protein signature on the samples following intensive washing and immunofluorescently-labelled TGFβ1 was detected on microfibre scaffolds. In vitro analysis demonstrated that GAG production was significantly enhanced in both the immobilised TGFβ1, and TGFβ1 in medium groups, compared to the control groups. This finding was further validated by the heightened production of GAGs and collagen type II, observed in histological sections.

We have demonstrated that APP-facilitated covalent immobilisation of TGFβ1 retains the growth factor's bioactivity and stimulates the differentiation of MSCs into the chondrogenic lineage. Our results also demonstrate that constructs with locally-immobilised TGFβ1 support neo-cartilage formation.

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Abstract **#272**

ENGINEERING CHONDROCYTE GEOMETRY FOR CONTROLLING CHONDROCYTE CYTOSKELETAL STIFFNESS

Marita Voelker$_P$[1], Jasmin C. Lauer[1,2], Mischa Selig[1,2], Bernd Rolauffs[1]

[1] G.E.R.N. Research Center for Tissue Replacement, Regeneration & Neogenesis, Dept. of Orthopedics and Trauma Surgery; Albert-Ludwigs-University of Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany

[2] Faculty of Biology, University of Freiburg, Schaenzlestrasse 1, D-79104 Freiburg, Germany

Corresponding author's email: berndrolauffs@googlemail.com

INTRODUCTION: The cytoskeleton and its stiffness are important for mechanotransduction, which integrates forces into molecular reactions. Osteoarthritis (OA) has been linked to changes in chondrocyte (CH) morphology and stiffness. We asked (i) whether OA CH stiffness can be controlled via engineering CH shape, and (ii) which geometric aspects of shape are effective in doing so.METHODS: Micro-printed fibronectin adhesion sites (MPs) shaped as H vs. circles and control areas were used for culturing human OA CHs overnight. Using live cell imaging with nano-indentation, CHs were indented above their nucleus (Chiaro Nanoindenter, OPTICS11; AxioObserver-Z1, Zeiss). The displacement was 10.000nm, the tip radius was 3µm, its ¬stiffness was 0.055N/m. Partial-least-squares regression (PLS) was used for finding fundamental relations between CH stiffness and shape. RESULTS: The CH Young's Modulus on controls was 869.58±62.77Pa (average±SE). Circular MPs led to a lower CH stiffness (small circles: 568.29±55.17Pa; large: 498.18±55.62Pa; ρ<0.05). The CH stiffness on small/large H-shaped MPs was in between values on control or H-shaped MPs (small H-shaped MPs: 807.99±78.42Pa; large: 675.79±54.43Pa; ρ>0.05). Geometrical aspects of CH shape such as area, major axis, aspect ratio, roundness, and solidity but not minor axis or circularity correlated with CH stiffness (ρ<0.05). Using PLS loadings for calculating the relative impact of shape on stiffness suggested that roundness (0.50), aspect ratio (0.50), and major axis length (0.35) were more associated with stiffness than area (0.32) and solidity (0.26).CONCLUSION: CH stiffness can be modulated by engineering cell shape. However, not all geometric aspects have equal effects on CH stiffness.

Abstract **#273**

3D BIOPRINTED DOUBLE NETWORK HYDROGELS TO SUPPORT CELL ENCAPSULATION

Adérito J. R. Amaral$_P$[1], Vítor M. Gaspar[1], Pedro Lavrador[1], João F. Mano[1]

[1] *University of Aveiro*

Corresponding author's email: aderito.amaral@ua.pt

Three-dimensional (3D) bioprinting has contributed significantly to advance engineered hydrogel platforms for tissue engineering and in vitro modelling [1]. Bioprinting technologies stand as ex vivo tools capable to create complex and spatiotemporally controlled tissue-like architectures using the appropriate living cells and biomaterials with tuneable physicochemical and mechanical properties. Of particular interest are marine-derived polysaccharides, which have received increasing attention owing to their abundance, reduced extraction cost, unique chemical and biological properties [2, 3]. This work focuses on the development of a novel dynamic bioink comprising boronic acid-functionalised laminarin and alginate for extrusion-based bioprinting of cell-laden gels through a double crosslinking approach that integrates covalent boronate esters formation and ionic interactions. The resultant hydrogel bioinks exhibit suitable printability and allowed for the fabrication of stable double network constructs with excellent tailorable rheologic properties, homogeneous cell distribution, and high cell viability for prolonged time periods in culture.
This simple and versatile platform is foreseen to accelerate the design of multifunctional 3D structures that will find uses in tissue engineering, delivery systems and in vitro disease modelling applications.

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Abstract **#274**

ENGINEERED GLYCO-BIOMATERIALS FOR 3D GLIOBLASTOMA BIOPRINTED MODELS

Lorenzo Rossi[P][1], Sofia Magli[1], Francesco Nicotra[1], Laura Russo[1]

[1] *University of Milano-Bicocca, Department of Biotechnology and Biosciences, Piazza della Scienza 2, 20126 Milan (Italy)*

Corresponding author's email: l.rossi58@campus.unimib.it

Glioblastoma is one of the most common and invasive primary brain cancers. Novel therapies are being investigated, but the research remains hindered by the limited reliability of currently employed preclinical models, which cannot mimic the complexity of tumor microenvironment. 2D cell cultures are effective but do not provide a reliable mimetic of extracellular matrix (ECM), which is a critical component in models for drug screening and cell biology studies.[1] ECM is a complex three-dimensional network representing the non-cellular part of tissues. The ECM composition differs between tissues and is involved in the maintenance of homeostasis and cell differentiation during tissue morphogenesis. Among the different ECM actors, the role of the glyco-microenvironment in the progression of glioblastoma is still poorly investigated due to the complexity and the dynamism of glyco-code. However, it is well known how glioblastoma shows a differential gradient of hyaluronic acid, associated with proteoglycans and GAGs, compared to the physiological brain tissue. This differential gradient results in both biomolecular and physical differences, playing an important role in the upregulation of cell migration. The design of new hybrid glyco-materials can quicken the characterization of glycosignature role in the progression of GBM. Here, glycoconjugate - ECM mimetics with tailored composition have been developed and characterized to investigate the role of glyco-microenvironment in GBM progression. Functionalized hyaluronic acid and protein-based components have been chemically crosslinked, resulting in a bioprintable hydrogel. The result is a 3D cell culture model, which exhibits multiple advantages over 2D models, mimicking in complexity the native counterpart.

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Abstract **#275**

MODEL-FREE BIOMECHANOLOGY OF BIOPRINTING AND BIOPRINTED MATERIALS

Michael Gasik[P][1,2], Yevgen Bilotsky[2]

[1] *Aalto University Foundation*

[2] *Seqvera Ltd.*

Corresponding author's email: michael.gasik@aalto.fi

Proper, consistent and correct biomechanical characterization is very important for novel 3D bioprinted constructs and scaffolds. Conventional approach foresees pre-selection of the material model and fitting experimental data to quantify properties in time and spatial scales. Here we present a new patented method of model-free assessment of invariant constitutive parameters of both bioprinted materials and the extrusion-based process with the same logic. This allows determination of e.g. biomaterials viscosity (without usual rheologic tests), stiffness, permeability, and other properties without a need of selection of material model and without use of complex transforms in frequency domain. Another advantage of the method is alignment of the testing parameters closer to intended clinical applications, making it more compliant with regulations such as MDR 2017/745 Annex I.

Abstract **#276**

REPAIR OF RAT CALVARIA DEFECT WITH STRONTIUM (SR2+)-DOPED POLYMERIC BRUSHITE CEMENT

Weiping Ren$_P$[1], Therese Bou-Akl[2], Paula Dietz[2], Bin Wu[2], Tong Shi[1], David Markel[2]

[1] *Wayne State University*

[2] *Providence Hospital*

Corresponding author's email: as7606@wayne.edu

An injectable polymeric brushite (PDCPD) cement was developed that is mechanically strong. The study aim was to evaluate bone healing with Strontium doped PDCPD (Sr- PDCPD) in a rat model. Rats were divided into: (1) Control (No PDCPD); (2) with PDCPD and (3) with 10%Sr-PDCPD (n=10 each). PDCPD was injected into 8-mm calvaria defects. Calvariae were harvested 12 weeks post-surgery. Bone healing was evaluated by µCT and bone histometric analysis. Data was analyzed by ANOVA. Results: Handling: Both PDCPD and Sr-PDCPD were injectable, moldable, and stable in vivo for up to 12 weeks. Histology: New bone formation was found with both cement groups. There was a close interaction between degrading cement fragments (particles) with surrounding newly formed bones. In vivo fluorescence labeling showed that much stronger fluorescent signals were observed in P-DCPD group (85%± 2.5) and in the Sr-P-DCPD group (60%± 2.5), as compared to control (<1%). In addition, no host inflammatory response to implanted cements was found. There were more cement fragments left in the Sr-PDCPD group than the P-DCPD group. µCT: The Sr doping significantly reduced the porosity of P-DCPD. It was difficult to define an appropriate threshold setting that could be used to quantify the dynamic interaction between new bone formation and cement degradation due to the high density of the implanted cements. Conclusion: P-DCPD is biocompatible, degradable and enhances new bone formation. The unoptimized Sr loading dose and the reduction of cement porosity may explain the inferior results of bone healing observed in Sr-PDCPD group.

Abstract **#277**

TOWARDS A NOVEL ALTERNATIVE TOXICITY MODEL WITH NORMOTHERMICALLY PERFUSED EX VIVO LIVERS BASED ON WHOLE PORCINE SLAUGHTERHOUSE ORGANS

Alicia Ruppelt[P][1,2], Marco Stijnen[1], Marco Rasponi[2], Melanie Krüger[1]

[1] *LifeTec Group BV*

[2] *Politecnico di Milano, Department of Electronics, Information and Bioengineering*

Corresponding author's email: a.ruppelt@lifetecgroup.com

Introduction

The development of highly predictive liver models for toxicity screenings is both important and challenging. Ex vivo liver perfusion has emerged in the last years as a promising tool to maintain organ functions and complexity. We here present a normothermic ex vivo liver perfusion model based on slaughterhouse material to reduce animal testing.

Methods

Procurement and treatment protocol of livers in the slaughterhouse was executed. Livers were attached to an extracorporeal perfusion circuit and reperfused at 39°C, applying pressures of 10 and 80 mmHg to veins and arteries, respectively. To assess liver function, we performed the indocyanine green (ICG) test, blood gas analysis, and quantified bile production and oxygen consumption.

Results

Blood gas values and pH were kept at physiological levels. Physiological flow rates of 864.3 ± 133.2 mL/min in the portal vein and 379.7 ± 92.8 mL/min in the hepatic artery were achieved without added vasodilators. Bile was produced continuously (6ml/h). The ICG functionality test resulted in an ICGt1/2 value of 12.86 min. Average oxygen consumption was 5 ml/min.

Conclusion

A normothermic perfusion model was developed to successfully perfuse an ex vivo liver obtained from a slaughterhouse. Functionality and viability were demonstrated by continuous bile production[1], which was confirmed by an ICGt1/2 result comparable to literature[2] and stable oxygen consumption[1]. Further analyses and circuit optimization will lead to extended perfusion and a versatile platform for interventional studies and drug treatment.

Acknowledgment

The project was funded by EU's Horizon 2020 research program under the Marie Skłodowska-Curie grant agreement No.860715.

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Abstract **#278**

PATTERNING APTAMER-FUNCTIONALIZED BIO-INKS FOR SPATIOTEMPORALLY CONTROLLED GROWTH FACTOR BIOAVAILABILITY

Deepti Rana[1], Vasileios D. Trikalitis[1], Vincent R. Rangel[1], Prasanna Padmanaban[1], Ajoy Kandar[1], Jeroen Rouwkema[1]

[1] *Department of Biomechanical Engineering, Technical Medical Centre, University of Twente, 7522NB Enschede, The Netherlands*

Corresponding author's email: d.rana@utwente.nl

In a developing tissue, spatiotemporally controlled growth factor bioavailability is crucial. The current growth factor delivery systems often focuses on growth factor immobilization or its coupling within the matrices (hydrogel), through which passive release rates and on-demand delivery could be achieved. However, it fails to adapt their release rates in accordance with the developing tissue. To this end, the present study employs oligonucleotides based aptamers for spatiotemporally controlled growth factor delivery. Aptamers are affinity ligands selected from DNA/RNA libraries to recognize proteins with high affinity and specificity.1 In the present study, authors have patterned aptamer-functionalized bio-inks using 3D bioprinting to study their potential for growth factor sequestering, programmable release and controlling vascular network formation. The aptamer-functionalized bio-inks were prepared via visible light induced photo-polymerization of gelatin methacryloyl (GelMA) and acrydite functionalized aptamers having sequence specific for binding to vascular endothelial growth factor (VEGF165). For studying the effect of growth factor (VEGF) bioavailability on vascular network formation, human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) were encapsulated within the bio-inks. The results obtained from ELISA assay confirmed the sequestration and triggered release of VEGF in response to complementary sequence (CS) addition after 4 days of culture. Interestingly, the immunostaining results showed vast CD31 positive endothelial network formation within the VEGF bound aptamer-patterned regions instead of GelMA regions. These observations altogether confirms the potential of aptamer-functionalized bio-inks in spatiotemporally controlling the bioavailability of VEGF via aptamers within the 3D printed constructs.

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Abstract **#279**

CRUCIAL GEOMETRICAL FACTORS OF CELL MORPHOLOGY IN RATIONALLY DESIGNING MICRO-PATTERNED ADHESION SITE REDUCTIONIST MODELS FOR CONTROLLING CHONDROCYTE PHENOTYPE

Jasmin C. Lauer[P][1,2], Marita Voelker[1], Mischa Selig[1,2], Bodo Kurz[3], Melanie L. Hart[1], Bernd Rolauffs[1]

[1] G.E.R.N. Research Center for Tissue Replacement, Regeneration & Neogenesis, Dept. of Orthopedics and Trauma Surgery, Albert-Ludwigs-University of Freiburg, Freiburg, Germany

[2] Faculty of Biology, University of Freiburg, Schaenzlestrasse 1, D-79104 Freiburg, Germany

[3] Institute of Anatomy, Christian Albrechts-University, Kiel, Germany

Corresponding author's email: jasmin.lauer@uniklinik-freiburg.de

Because chondrocyte (CH) morphology is associated with type I & II collagen expression levels [1], CH expression can be controlled by engineering CH shape using micro-patterned adhesion sites (MPs). The aim was to test rationally designed MP models that vary geometric aspects such as area, width, length, aspect ratio, circularity, and solidity to control CH cytoskeleton and phenotype.

MP reductionist models were designed as fibronectin-coated circles/ellipses with increasing area, circles with increasing prong numbers but constant area (star shapes), and non-solid MPs. MPs were used for human CH culture, ddPCR for quantifying mRNA expression, and fluorescence microscopy and nano-indentation for assessing the cytoskeleton. Partial least square (PLS) regression was used to analyze the impact of geometry on CH characteristics.

MPs altered CH shape, all mRNA expression levels ($p<0.05$), and cytoskeletal properties such as actin intensity, distribution and stiffness. Subsequent PLS analyses revealed COL2A1, the COL2A1/COL1A2 ratio, ACAN, SOX9 were positively regulated by solidity and COL1A2 by aspect ratio, whereas solidity, and, to an even larger extent, circularity negatively regulated COL1A2 expression. Systematically varying MP area of sets of circles or ellipses did not modulate mRNA expression levels in linear dose-responses. Instead, maximizing the resulting expression levels was achieved by using defined ranges of MP area. When using star-shaped MPs, defined ranges of circularity and solidity maximized the resulting expression levels similarly.

Thus, specific combinations of geometric features - and not a specific feature alone - are needed for inducing high mRNA expression levels of chondrogenic genes in human CHs.

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Abstract **#280**

ENDOTHELIAL PROGENITOR CELLS ADHERE TO LAMININ-CONJUGATED NANOCELLULOSE CONSTRUCTS - A FACILITATOR OF VASCULARIZATION IN ENGINEERED TISSUE

Karin Säljö[1], Susann Li[2], Peter Apelgren[1], Kristin Oskarsdotter[3], Sanna Sämfors[3], Paul Gatenholm[3], Stina Simonsson[2], Lars Kölby[1]

[1] Department of Plastic Surgery, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden and Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden

[2] Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicin, Sahlgrenska Academy, Gothenburg, Sweden

[3] 3D Bioprinting Centre, Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, Sweden.

Corresponding author's email: lars.kolby@surgery.gu.se

Lack of vascularization is a major limitation for all engineered tissue. One possible way to facilitate formation of blood vessels could be recruitment of endothelial cells to bioactive surfaces.

In the present work, tunicate nanocellulose hydrogel surfaces were oxidized with sodium periodate and bioconjugated with laminin produced from mouse Engelbreth-Holm-Swarm sarcoma cells. A population of CD34+(CD45-) endothelial progenitor cells (EPCs) were sorted from human Stromal Vascular Faction (SVF) by Fluorescence Activated Cell Sorting (FACS) and cultured in vitro for 13 days in ECGM MV2 medium. EPCs (passage 1) was incubated for 3 days with the bioconjugated surfaces. For comparison, unsorted SVF was also applied. Unconjugated surfaces served as negative controls. Cell adhesion was evaluated by confocal laser microscopy using immunofluorescent CD 34 antibodies and DAPI for nuclear staining.

Surfaces bioconjugated with laminin showed extensive adhesion of CD34+ cells from SVF whereas laminin-free surfaces showed no adhesion of these cells. Direct application of SVF also allowed adhesion of CD34+ cells but to a lesser extent than the EPC population.

In summary, here a method to increase functionality of nanocellulose hydrogel constructs by bioconjugation with laminin improved adhering of EPCs from SVF. This is a possible step towards autologous vascularization of 3D bioprinted constructs.

Keywords

Stromal Vascular Fraction; endothelial progenitor cells; nanocellulose

Abstract **#282**

TOWARDS THE BIOHYBRID LUNG: OPTIMISATION OF ENDOTHELIAL CELL SEEDING DENSITY ON HOLLOW FIBRES OXYGENATORS

Ariana Mónica Silva Peredo[1,2], Michael Pflaum[1,2], Daniele Dipresa[1,2], Sotiria Toumpaniari[3], Bettina Wiegmann[1,2], Axel Haverich[1,2], Sotiris Korossis[P][1,2,3]

[1] Lower Saxony Centre for Biomedical Engineering Implant Research and Development, Hannover Medical School, Hannover, Germany

[2] Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany

[3] Cardiopulmonary Regenerative Engineering (CARE) Group, Centre for Biological Engineering, Loughborough University, United Kingdom

Corresponding author's email: S.Korossis@lboro.ac.uk

Chronic obstructive pulmonary disease is one of the main causes of death worldwide. Currently, lung transplantation is the only currently available treatment; however, donor shortage and organ rejection represent major limitations. The current short-term alternative, the extracorporeal membrane oxygenators (ECMO), comprises highly-hydrophobic hollow fibres (HFs) made of polymethylpentene. Thrombogenicity and associated clotting of the ECMO limits its operation to about 30 days. Endothelialisation of the HFs in the ECMO has been proposed to enhance hemocompatibility and decrease thrombogenicity of the device. This study was aimed at optimising the seeding of the HFs of the oxygenator with endothelial cells (ECs).Human cord blood endothelial cell (HCBEC) adhesion was assessed on rheoparin®-coated and rheoparin®-fibronectin-coated HF mesh samples (3×2.6 cm) from commercially-available oxygenators (Xenios, Hilite 2400 LT). The HF mesh samples were mounted in custom-made frames. Seeding was carried out dynamically (4 h, 1 rpm rotation) on falcon tubes filled with cell suspension at defined cell densities (1.72E4, 3.43E4, 6.88E4, 8.56E4, 1.03E5 and 1.20E5 cells/cm2). After 2 days of static culture in culture dishes, the samples were assessed through live/dead staining. Rheoparin® alone did not allow HCBEC adhesion. Rheoparin®-fibronectin-coated samples demonstrated improved cell adhesion. Confluent HCBEC coverage was achieved around the HFs coated with rheoparin®-fibronectin at an optimum seeding density of 1.03E5 cells/cm2. These results provide the starting point for scaling up the seeding strategy for endothelialisation of the whole HF stack in commercially available oxygenators.

Abstract **#283**

MICROFLUIDIC GENERATION OF IMMUNOPROTECTIVE AND ENZYMATICALLY CROSSLINKED ULTRA-THIN POLYETHYLENE GLYCOL-TYRAMINE MICROCAPSULES FOR MINIMALLY INVASIVE DELIVERY OF BETA CELLS

Nuno Araújo-Gomes[P][1], Barbara Liszka[1], Sebastiaan R. van Loo[1], Suzanne Nijhuis[1], Tom Kamperman[1], Alexandra M. Smink[2], Bart de Haan[2], Marcel Karperien[1], Jeroen Leijten[1]

[1] Department of Developmental BioEngineering, TechMed Centre, University of Twente, the Netherlands

[2] Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen , Groningen , The Netherlands

Corresponding author's email: n.m.araujodacunhagomes@utwente.nl

Non-autologous beta cells can be protected from the patient's immune system using biomaterials such as immunoprotective microcapsules. Although several material formulations have been developed, clinical translation of immunoprotective microcapsules is challenged by their non-permanent nature, fibrous capsule formation upon implantation, and low production rates. We here report the development of a novel, immunoprotective biomaterial that allows ultra-high throughput microfluidic production of beta-cell containing microcapsules.

Enzymatically crosslinkable polyethylene-glycol 8 arm–tyramine (PEG-TA) conjugates were synthesized in a two-step reaction. Microfluidic droplet generation was optimized for production of hollow PEG-TA microgels using a delayed outside-in crosslinking strategy based on horseradish peroxidase and cytocompatible levels of hydrogen peroxide. Production rates and regimes, permselectivity, cytocompatibility, immune reactivity, glucose responsiveness were analyzed in vitro, and fibrous capsule formation was analyzed in vivo.

We were able to produce monodisperse microcapsules with a diameter of 200 µm and a shell thickness of 20 µm using conventional microfluidics and in ultra-high throughput using in-air microfluidics. Incubating microcapsules with FITC-IgG of different Mw revealed that molecules from 150 kDa onwards were unable to diffuse into the microcapsules. Exposing microcapsules to monocytes and whole blood did not result in macrophage activation or cytokine release based on immunofluorescence and multiplex ELISA. Histological analysis revealed minor fibrotic structures surrounding the particles, most likely due to the micromaterials' geometry. Microencapsulated beta-cells remained viable, metabolically active, and responsive to glucose.

High throughput production of immunoprotective PEG-TA microcapsules using microfluidic droplet generation is a promising novel combinatorial approach for delivery and protection of non-autologous beta cells.

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Abstract **#284**

EFFECT OF THE PORE SIZE OF ELASTOMERIC POLY(ESTER-URETHANE-UREA)-BASED SCAFFOLDS DEDICATED TO SOFT TISSUE REGENERATION ON CELL RECRUITMENT, TISSUE INVASION AND TISSUE ORGANIZATION

Geraldine ROHMAN$_P$[1], Wassim Manhal[2], Karim Senni[3], Carole Chakar[2], Didier Lutomski[1], Ronald Younes[2], Sylvie Changotade[1]

[1] *Université Sorbonne Paris Nord, URB2I UR 4462*

[2] *Saint Joseph University of Beirut, Faculty of Dental Medicine*

[3] *Ecole de biologie industrielle, Cergy*

Corresponding author's email: geraldine.rohman@univ-paris13.fr

Introduction

A previous study has shown that elastomeric poly(ε-caprolactone-urethane-urea) (PEUU)-based scaffolds display a good biocompatibility and biointegration in an in vivo bone healing model [1]. However, the structural and physico-chemical characteristics of scaffolds have to be optimized when dedicated to soft tissue regeneration. The objective of this work was to study the effect of PEUU scaffold pore size on cell infiltration and migration and tissue reconstruction in a subcutaneous pockets model in rats.

Materials and method

A homologous series of PEUU scaffolds were obtained by cross-linking a high internal phase emulsion [2]. Scaffolds were evaluated in vivo by subcutaneous implantation alongside the dorsal midline of rats. Seven day after implantation, rats were sacrificed and the implanted scaffolds and the surrounded tissues were biopsied and prepared for histological studies.

Results and Discussion

The PEUU scaffolds were developed with differences in pore size ranges and mean pore diameters (490, 715, and 1075 µm), but with no significant variation in others parameters. Histological analysis showed that fibroblast-type cells were able to migrate within the porous structure of all scaffolds. The tissue invasion seemed to be more important when the scaffold pore size increased. However for smaller pore size, the connective tissue appeared more structured, dense and fibrillar with presence of newly formed vessels. The study provides evidence that mean pore diameters much larger that those recommended by the literature for soft tissue engineering can be used. The study also demonstrated that scaffold pore diameters had a strong influence on tissue structuring.

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Abstract **#285**

VALIDATION OF A CUSTOM-MADE BIOREACTOR FOR PERFUSION CULTURE AND LONGITUDINAL MONITORING OF BIOENGINEERED WHOLE LIVERS WITH HEPATOCYTES

Omolola Ajayi[1], Lisa Sassi[1], Sara Campinoti[1], Claire McQuitty[1,2], Dipa Natarajan[1], Riccardo Rayan Siena[1], Alessandro Pellegata[3], Paolo De Coppi[3], Sara Mantero[4], Shilpa Chokshi[1,2]

[1] *Institute Of Hepatology London, Foundation for Liver Research*

[2] *Faculty of Life Sciences & Medicine, King's College London*

[3] *Stem Cells and Regenerative Medicine, Great Ormond Street Institute of Child Health, UCL, London, UK*

[4] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milan, Milan, Italy*

Corresponding author's email: luca.urbani@researchinliver.org.uk

Bioengineered liver tissue can recapitulate the hepatic microenvironment for disease modelling. Bioreactors overcome challenges in engineering and culturing tissue constructs. However, novel bioreactors are needed to support long-term culture of bioengineered constructs allowing non-invasive longitudinal monitoring of culture parameters and cell behaviour. In this study we designed and validated a custom-made bioreactor for long-term perfusion 3D culture of whole liver constructs with traceable hepatocytes.

Whole scaffolds were generated by perfusion decellularisation of rat livers. The bio-inert and chemical resistant custom-made bioreactor was produced in nylon. Luc+HepG2 cells or primary human hepatocytes (PHH) were perfusion-seeded into scaffolds and cultured in static vs dynamic conditions using the bioreactor with a programmable syringe pump, providing continuous unidirectional flow. The bioreactor was designed to provide non-invasive monitoring of perfusion parameters, media sampling and evaluation of cellular rearrangements and growth measured with bioluminescence; cell function analysis was performed with immunofluorescence and ELISAs.

Luc+HepG2 viability, distribution, and function in bioengineered livers were monitored using bioluminescence for 11 days. Bioreactor-perfused scaffolds had significantly higher cell viability, homogenous distribution, and functionality in comparison to static conditions. PHH cultures were followed for up-to 30 days, showing remarkably higher cell viability and function in bioreactor culture compared to static conditions: albumin secretion, expression of HNF4α, CYP3A4 and FOXA2 and urea production were increased in perfusion-culture vs static.

The bioreactor novel design permitted longitudinal monitoring of whole-liver constructs, supporting PHH viability, metabolism, media sampling and maintaining sterility, making it well suited for liver tissue engineering and potentially culture multicellular constructs.

Keywords

decellularization; bioengineered liver; bioluminescence

Abstract **#286**

LIGHT-ASSISTED ELECTROSPINNING OF CORE-SHELL P(NIPAAM-CO-NIPMAAM) HYDROGEL-BASED NANOFIBERS FOR THERMALLY SELF-REGULATED DRUG DELIVERY

Chiara Rinoldi[P][1], Sylwia Pawłowska[1], Paweł Nakielski[1], Yasamin Ziai[1], Olga Urbanek[1], Tomasz Aleksander Kowalewski[1], Filippo Pierini[1]

[1] Department of Biosystems and Soft Matter and Laboratory of Polymers and Biomaterials, Institute of Fundamental Technological Research, Polish Academy of Sciences. Warsaw 02-106, Poland

Corresponding author's email: crinoldi@ippt.pan.pl

Recently, stimuli-responsive drug delivery systems are gained researchers' attention, targeting sustained and controlled drug release in order to deliver the requested active molecule dose, while avoiding unwanted cell uptake. The adjustment of drug release at different stages of therapy is highly desirable because changes and related timing are crucial factors in most biological processes. In this frame, several nanostructured biomaterials have been developed to meet those requirements. However, only materials able to release drugs over long term were obtained, without taking into account the optimization of the bioactive molecule secretion level at the first stage after implantation.

In this work, a co-axial electrospinning technique is used to fabricate core-shell fully cross-linked copolymer poly(N,N-isopropylacrylamide-co-N-isopropylomethacrylamide) (P(NIPAAm-co-NIPMAAm) hydrogel-based nanofibers, as thermal-responsive drug delivery system. The custom-made electrospinning apparatus enables the in situ cross-linking of P(NIPAAm-co-NIPMAAm) hydrogel core into a nanoscale confined PLCL shell, hence improving the electrospun nanofiber drug dosing process by reducing its provision and allowing a self-regulated release control.

Furthermore, the thermoresponsivity of the system also acts as drug release self-regulating factor, revealing temperature dependent drug delivery kinetics driven by an optimized temperature-controlled desorption. Thus, tuned thermoresponsive properties of the material lead to a decrease of the drug delivery at early stages while long-term release is guaranteed, hence showing that the system is a promising candidate as a "smart" drug delivery platform.

Acknowledgments

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Keywords

core–shell nanofibers; thermo-responsive hydrogels; smart drug delivery

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Abstract **#287**

STUDY OF THE LIVER FIBROGENIC MICROENVIRONMENT IN A BIOENGINEERED MODEL OF CHRONIC LIVER DISEASE

Sara Campinoti[P][1,2], Omolola Ajayi[1], Lai Wei[1,2], Sveva Fagiolino[1,2], Lorenzo Caciolli[1], Dipa Natarajan[1,2], Shilpa Chokshi[1,2], Luca Urbani[1,2]

[1] *Institute of Hepatology, Foundation for Liver Research, London, UK*

[2] *Faculty of Life Sciences & Medicine, King's College London, UK*

Corresponding author's email: luca.urbani@researchinliver.org.uk

Chronic liver disease (CLD) poses a significant health burden world-wide. Its progression is characterized by chronic injury, inflammation, and fibrosis, caused by the accumulation of extracellular matrix (ECM) by activated hepatic stellate cells (HSC). These mechanisms of chronic inflammation remain poorly understood, mainly due to the inability of conventional cell culture models to mimic the liver microenvironment.

Here, we used bioreactor-based bioengineered systems to culture hepatic cells in 3D conditions as a tool to examine cell-ECM crosstalk in the context of CLD.

We isolated human HSCs from total and partial hepatectomy and provided an in-depth cell characterization in comparison to commercially available HSC lines.

Cells were cultured in 3D systems generated using decellularized human liver ECM-scaffolds from normal and fibrotic livers. 3D cultures were maintained using a custom-made confined-perfusion bioreactor. HSCs were also co-cultured with primary hepatic endothelial cells. HSC activation and cell phenotypes were analysed.

Phenotypic and gene expression analysis of seeded scaffolds were performed using immunofluorescence and qPCR.

Culturing HSCs in 3D ECM-scaffolds reduced cell activation in comparison to standard 2D cultures, possibly due to change in substrate stiffness. Primary HSC activation and ECM production was achieved within the 3D construct with the addition of profibrogenic factor TGFβ. Cell-cell and cell-ECM crosstalk produced various levels of HSC quiescence/activation, based on the fibrosis score of ECM-scaffold used.

In conclusion, confined perfusion supported the culture of liver constructs populated with primary human hepatic cells, generating a dynamic 3D model useful to investigate the liver microenvironment in CLD.

Keywords

decellularized extracellular matrix; liver disease; perfusion bioreactor

Abstract **#288**

COMPUTATIONAL MODELLING OF CELLS EXTRUSION IN BIOPRINTING

Gianluca Santesarti[P][1], Michele Marino[1], Francesco Viola[2], Roberto Verzicco[2,3], Giuseppe Vairo[4]

[1] *Department of Civil Engineering and Computer Science DICII, University of Rome Tor Vergata, Rome, Italy*

[2] *Gran Sasso Science Institute, L'Aquila, Italy*

[3] *Department of Industrial Engineering, University of Rome Tor Vergata, Rome, Italy*

[4] *Department of Civil Engineering and Computer Science, University of Rome Tor Vergata, Rome, Italy*

Corresponding author's email: gianluca.santesarti@uniroma2.it

The final outcome of extrusion-based bioprinting procedures is highly affected by a number of process variables, such as bio-ink properties, nozzle diameter and extrusion pressure. Introducing in silico (computational) models as an integral part of the bioprinting design pipeline would allow to increase innovation, productivity, scalability and robustness. To date, simulations are not employed in the field since theoretical and computational models for describing the physicochemical mechanisms occurring during/after bioprinting are not available or refined enough for giving quantitative predictions.
The objective of this work is to develop a computational model for the extrusion of non-Newtonian shear-thinning hydrogels with a cellular component. In fact, cell densities affect both the extrusion flow rate and the final cell viability. The aim is to explicitly describe the extrusion of cells through the nozzle, predicting the cellular membrane stresses during the process, and then the onset of cell damage or apoptosis. The computational framework has to account for both multiscale dimensionality and fluid-structure interactions. In fact, cell diameter is significantly smaller than the extruder and the final construct but is comparable with nozzle size. Moreover, cells significantly deform when moving within the hydrogel. Global-local strategies are then employed and coupled with immersed boundary formulations of the FSI problem. The proposed model is a first step towards a predictive tool for the optimal setting of process variables such to guarantee a delicate balance between the biological request of high cell densities and the engineering constraint of bio-inks printability.

Abstract **#289**

CEREBRAL ORGANOIDS FOR CHRONIC PAIN: UNRAVELING THE CELLULAR AND BIOLOGICAL MECHANISMS CORRELATING BRAIN PLASTICITY AND ANALGESIC PROPERTIES OF MESENCHYMAL STEM CELLS' SECRETOME

Aline Marie Fernandes[P][1,2], Jonas Campos[1,2], Nuno Silva[1,2], António Salgado[1,2]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho*

[2] *ICVS/3B's – PT Government Associate Laboratory*

Corresponding author's email: alinemarie.fernandes@med.uminho.pt

Chronic pain (CP) is one of the most frequent medical condition that affects 20% of adult population limiting their life and work activities. Over 90% of CP patients receive treatment based on opioids which increased the prevalence of opioid use disorder, resulting in a national crisis for public health, social and economic wellbeing.

Opioid drugs act through MOR, DOR and KOR opioid receptors activation. MOR, the main target and most studied receptor is highly distributed in areas related to pain perception and sensorimotor integration and when activated produces analgesia. Our preliminary results showed the presence of MOR, DOR and KOR in Cerebral Organoids (CeO) with higher MOR expression. CeO are self-organized, functional and multicellular 3D structure that resemble the brain. These results suggest CeO as a powerful tool to understand opioid receptors modulation and brain plasticity.

Chronic opioid consumption involves alterations in neurotransmission, dendritic branching, spine density and pro-apoptotic proteins at reward circuit regions. To overcome these issues, efforts have been made to find an alternative treatment for CP. Recent interest on bioactive molecules secreted by Stem Cell, known as secretome has increased. Secretome include a broad spectrum of growth factors, cytokines and vesicular fraction already described effective in pain models. Nevertheless, to our knowledge, no study regarding central pain mechanisms at brain level were made using secretome. Recent result from our lab showed modulation of opioids receptors when CeO were incubated with secretome. Herein, we aim to prove the prolonged analgesic effect of secretome, without negative outcomes of opioids.

Abstract **#290**

DESIGN OF BIOMATERIALS TO MODULATE INFLAMMATION

Kam Leong$_P$[1]

[1] *Columbia University*

Corresponding author's email: kam.leong@columbia.edu

Inflammation plays an important role in responding to danger signals arising from damage to our body and in restoring homeostasis. Controlling the inflammatory response is a major strategy in managing diseases such as cancer, autoimmunity, and wound healing. While conventional drug therapies are the norm in tackling inflammation, biomaterials are increasingly proposed to join the battle. Through drug delivery strategies, biomaterials potentiate the efficacy of anti-inflammatory drugs by improving bioavailability and diminishing side-effects. Applied in inhibitory or scavenging strategies, they reduce inflammation by removing the pro-inflammatory factors. For instance, the scavenging approach may be applied to inflammatory diseases such as rheumatoid arthritis, psoriasis, multiple sclerosis and systemic lupus erythematosus, which are increasingly linked to inappropriate and chronic activation of inflammatory cells. A central event in the pathogenesis of these diseases appears to be an aberrant activation of innate immune sensors, most prominently the Pattern Recognition Receptors (PRRs), by nucleic acids that are released from dead and dying cells. In this presentation, I will discuss the application of nucleic acid-binding polymers in the configuration of either soluble polycation or cationic nanoparticle to scavenge these nucleic acids as a molecular strategy to combat inflammation.

Abstract **#291**

DEVELOPMENT OF A NANOFIBROUS PLGA WRAP TO DELIVER PPARΓ AGONISTS LOCALLY IN A CONTROLLED RELEASE MANNER TO TREAT PERIPHERAL NERVE INJURY

Melissa Rayner[P][1,2], Alessandra Grillo[1], Gareth Williams[1], Essam Tawfik[1], Tianqi Zhang[1], Charitini Volitaki[1], Duncan Craig[1], Jess Healy[1], James Phillips[1]

[1] *University College London, Gower Street, London, UK.*

[2] *MRC Prion Unit at UCL, Institute of Prion Diseases, Cleveland Street, London, UK.*

Corresponding author's email: melissa.rayner.14@ucl.ac.uk

Despite advances in microsurgical techniques to repair peripheral nerve injuries (PNI), full regain of function is rarely achieved. Neuron growth rate is limited to ~1 mm/day meaning long periods of time can elapse before the neurons reach their target organs. The lack of nerve stimulation leads to atrophy of the muscle over time causing irreversible wasting. Therefore, there is a unmet clinical need to accelerate nerve regeneration. Despite drug candidates demonstrating beneficial effects on nerve regeneration rate in preclinical models, there are currently no drugs routinely used in clinical practice. The main hindrance of such drugs is the side effects associated with sustained systemic administration. A local controlled release drug delivery system could potentially address this challenge, particularly through the use of drug-loaded biomaterials that could be implanted at the repair site during the microsurgical repair procedure. This study tested this concept by delivering PPARγ agonists from nanofibrous polylactic-co-glycolic acid (PLGA) locally in a controlled manner in a rat sciatic nerve injury model. Following characterisation of release parameters in vitro, PLGA loaded with ibuprofen sodium or sulindac sulfide were used in vivo to treat a nerve crush injury in rats. The results demonstrated that ibuprofen sodium, but not sulindac sulfide caused an increase in neurites in the distal nerve and improved functional recovery. This study has shown for the first time that the local delivery of ibuprofen using biomaterials has beneficial effects on neuron regeneration. This will underpin future development of drug-loaded biomaterials suitable for clinical translation to treat PNI.

Abstract **#292**

STEM CELLS IN ENGINEERED NEURAL TISSUE REPAIR LONG GAP PERIPHERAL NERVE INJURIES

Melissa Rayner[P][1,2], Adam Day[1,2], John Sinden[2,3], James Phillips[1,2,3]

[1] *School of Pharmacy, UCL, London, UK.*

[2] *UCL Centre for Nerve Engineering, London, UK.*

[3] *Glialign Ltd, UCL School of Pharmacy, London, UK.*

Corresponding author's email: melissa.rayner.14@ucl.ac.uk

Following a peripheral nerve injury the nerves have capacity to regenerate naturally, however, in a gap injury surgical repair is required. Current microsurgical interventions use an autograft as the clinical gold-standard therapy for gap repair, however, there are challenges in achieving full recovery and limitations associated with tissue availability and donor-site morbidity. Tissue engineering provides an opportunity to overcome these limitations by building a construct from therapeutic cells and biomaterials to artificially recreate the autograft. In this study engineered neural tissue (EngNT) was manufactured using human neural stem cells (CTX0E03) to bridge a critical-length (15 mm) sciatic nerve gap injury in athymic nude rats. The ability of EngNT-CTX to enhance regeneration and functional recovery was compared to an autograft. A range of outcome measures that assessed neuronal regeneration and functional recovery were investigated at 8 and 16 weeks post-repair.

Histological analysis showed the number of neurons (motor + sensory) detected in the distal nerve stump was equivalent in both groups at 16 weeks. At the 8 week time point there were more sensory neurites detected in the autograft group than the EngNT-CTX group, indicating increased sensory sprouting, although motor neuron numbers were equivalent. These results were consistent with the electrophysiology, which showed EngNT-CTX restored electrophysiological nerve conduction and functional reinnervation of downstream muscles to an equivalent or greater extent than the autograft at both time points.

In conclusion, this study provides evidence that in a critical-length preclinical peripheral nerve injury model EngNT-CTX demonstrates equivalent performance to an autograft.

Abstract **#293**

DESIGNING BIOMATERIALS THAT RESTORE TISSUE FUNCTIONALITY AND INTEGRATE FULLY IN VIVO

Nazia Mehrban[P][1], Catalina Pineda Molina[2], Lina M Quijano[2], James Bowen[3], Scott A Johnson[2], Joseph Bartolacci[2], Jordan T Chang[2], David A Scott[4], Derek N Woolfson[4], Martin A Birchall[1], Stephen F Badylak[2]

[1] *University College London*

[2] *University of Pittsburgh*

[3] *The Open University*

[4] *University of Bristol*

Corresponding author's email: n.mehrban@ucl.ac.uk

Introduction: Tissue engineering is a balance between the functional restoration of tissue and the reconstruction of matrix to allow full integration with the surrounding healthy tissue. Bespoke biomaterials offer the ability to do both by fine-tuning the chemical and physical characteristics of the material.

Method: We present de novo alpha-helical peptides, designed and manufactured using standard solid-phase synthesis protocols. The peptides are purified using reversed-phase HPLC and masses confirmed through mass spectrometry. The peptides form novel hydrogels that are chemically tuned using click chemistry methodology and can be used as in vitro 3-dimensional structures or injected in vivo.

Result: The alpha-helical hydrogels encourage cell attachment, directional migration and promote functional differentiation in a variety of cell types. Hydrogel stiffness is modifiable and can be used to create an injectable scaffold, which over 28 days increases cell infiltration at the wound interface. No foreign body reaction or encapsulation is observed during that time. In a rat abdominal model we also note an upregulation of myogenic differentiation markers as well as the promotion of an anti-inflammatory environment. After 28 days the hydrogel-tissue interface is not discernible, indicative of full integration.1

Conclusion: Designing peptides using a bottom-up approach allows control over the final material. These alpha-helical hydrogels offer a chemically and physically tuneable environment that can be easily manipulated to suit different tissue types. They offer the ability to promote functional restoration of tissue while also integrating fully with healthy tissue. These peptide-based materials offer great potential towards unmet clinical needs.

Keywords

Peptides; De novo; Hydrogels

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Abstract **#294**

MULTIVARIABLE MECHANO-BIOCHEMICAL PERTURBATIONS OF DEVELOPING VASCULAR NETWORKS WITHIN CHICK CHORIOALLANTOIC MEMBRANE

Prasanna Padmanaban[P][1], Nasim Salehi-Nik[1], Jeroen Rouwkema[1]

[1] *Department of Biomechanical Engineering, Technical Medical Centre, University of Twente, The Netherlands*

Corresponding author's email: p.padmanaban@utwente.nl

Spatiotemporal organization of micro and macrovascular networks within engineered tissues can be controlled using mechanical and chemical cues. Recent studies have shown that the local geometric shapes [1], external fluid flows [2] and growth factor signals [3] influence the development, organization, and orientation of sprouting blood vessels. Nevertheless, the physiological relevance is unknown and yet this has not been tested directly on the in vivo culture platforms. Here we developed two types of PDMS based ex ovo culture systems that allow us the long term observation of developing vascular networks within chick CAM and compatible with multivariable mechano-biochemical perturbations. We perturb the developing vascular networks using mechano-biochemical signals such as geometric shapes, external fluid flows, and VEGF concentrations in three different ex ovo models. Time-lapse image analysis showed the differences in the vascular organization as well as variation in the vascular response to the effect of these signals. Furthermore, we performed quantification metrics on vessel diameter, vessel length, and sprouting percentage on the above-mentioned test conditions, in comparison to the control samples. Our strategy implies the possibility of adding multiple mixed combinations of mechanical and chemical signals within a single in vivo culture system and offers to probe local as well as global structural changes over the development. We anticipate our culture system to be a foundation for directing in vivo vascular network development, remodeling, and organization.

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Abstract **#295**

EFFECT OF PORE DIRECTIONALITY OF BMP-2 AND BMP-2/VEGF-LOADED COLLAGEN SCAFFOLDS ON BONE FORMATION IN VIVO.

Miguelangel Moncayo Donoso[P][1], Gustavo Rico Llanos[2], Diego Alexander Garzón Alvarado[3], Jose Becerra Ratia[4], Rick Visser[4], Marta Fontanilla Duque[3]

[1] *Universidad Nacional de Colombia - Universidad de Málaga*

[2] *Centro andaluz de nanomedicina y biotecnología (BIONAND)*

[3] *Universidad Nacional de Colombia*

[4] *Universidad de Málaga*

Corresponding author's email: mmoncayod@unal.edu.co

The scarcity of donor tissue has led to the development of tissue engineering products that replace bone grafts. However, the development of bone tissue engineering products that mimic the microstructural characteristics of native bone continues to be challenging. In this work, we developed and characterized type I collagen scaffolds with two different pore orientations, unidirectional pores (UC) characteristic of cortical bone, and multidirectional pores (MC) characteristic of trabecular bone. Microstructural, physicochemical, mechanical, and biological characteristics were evaluated in vitro. We also assessed the scaffolds' ability to support bone growth when loaded with BMP-2 or BMP-2 plus VEGF in an ectopic rat model. Both scaffolds had porosity above 90% and interconnected pores. The MC scaffolds' tensile strength, elasticity, and liquid sorption capacity were significantly higher than the ones found in UC scaffolds. Culture of rat bone marrow mesenchymal stem cells demonstrated that scaffolds support cell adhesion, growth, and osteoblastic differentiation in the presence of BMP-2. In vivo evaluation of scaffolds loaded with BMP-2 or BMP-2 plus VEGF demonstrated they promoted bone formation ectopically. Histological and calcium analyses content showed pore directionality impact osteogenic differentiation because UC scaffolds loaded with BMP-2 plus VEGF contained significantly more bone than the MC loaded with these same factors. Overall, longitudinal bone formed in the UC scaffolds, and random bone formed in the MC scaffolds indicated bone deposition is guided by scaffolds' pore directionality.

Keywords

Bone tissue engineering; pore directionality; type I collagen

Abstract **#297**

EFFECT OF KIDNEY STEM CELLS ON BIOCHEMICAL ANALAYSIS AND SMAD2/3 PHOSPHORYLATION IN RATS WITH DIABETIC NEPHROPATHY

Zeinab Rafieeₚ[1], Mahmoud Orazizadeh[2,3], Esrafil Mansouri2[2,3]

[1] *Cellular & Molecular Research Center, Ph.D student of Anatomical sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[2] *Cellular & Molecular Research Center, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[3] *Department of Anatomical Sciences, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences,Ahvaz, Iran*

Corresponding author's email: Zeinabrafiee@yahoo.com

Introduction: Mesenchymal stem cells (MSCs) were proposed as a critical therapeutic candidate in diabetic nephropathy (DN). Renal stem cells as a source for repairing are controversial. The purpose of the present study was to evaluate the effect of kidney rat stem cells on DN.

Materials and methods: After separation of renal stem cells from rat kidney, the surface stem cell markers were assessed by flow cytometry analysis. To establish the diabetic nephropathy rat model STZ(60mg/kg) was used. The cells were injected to experimental groups via tail vein (2×106cells/rat). Biochemical and histological parameters were evaluated in order to determining the impact of stem cells on kidney structure. Phosphorylation of Smad2/3 two weeks after induction of early diabetic nephropathy was evaluated by using standard western blotting.

Result: The cells significantly reduced blood nitrogen (BUN), serum creatinine(Scr) and 24 urinary proteins. The phosphorylation of smad2 and smad3 significantly down-regulated in diabetic group. PAS staining showed in the presence of adult kidney stem cells histopathological changes were improved.

Conclusion: Adult kidney stem cells may be a candidate for treatment of early DN to improve the kidney function and regenerating kidney tissues in DN rats.

Keywords

stem cell therapy; Diabetic Nephropathy; kidney stem cells

Abstract **#298**

DEVELOPMENT OF A BIOABSORBABLE IMPLANT FOR REGENERATION OF ADIPOSE TISSUE

Shuichi Ogino[P][1], Michiharu Sakamoto[2], Takashi Nakano[2], Sunghee Lee[2], Hiroki Yamanaka[2], Yusuke Kambe[3], Atsuhiro Arakawa[1], Junko Okano[1], Tetsuji Yamaoka[3], Yoshihisa Suzuki[1], Naoki Morimoto[2]

[1] *Department of Plastic and Reconstructive Surgery, Shiga University of Medical Science*

[2] *Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyoto University*

[3] *Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute*

Corresponding author's email: sogino12@belle.shiga-med.ac.jp

Breast reconstruction utilizes autologous fat injection, flap reconstruction, and silicone breast implants. However, these methods have poor take rate, donor site morbidity, and the occurrence of breast implant-associated anaplastic large cell lymphoma. Adipose tissue has been regenerated by combining scaffolds, growth factors (GFs), and/or adipose tissue-derived stromal cells (ASCs). However, the safety of GFs or ASCs after mastectomy has not been confirmed. We reported that a poly L-lactic acid (PLLA) mesh implant containing a collagen sponge (CS) maintained the internal space and the formed adipose tissue in vivo for up to 12 months. We developed a PLLA capsule implant and compared adipose tissue formation at 12 and 24 months after implantation between the PLLA mesh with CS implant and the PLLA capsule implant with or without CS in rabbit models.

All implants maintained the internal space at 12 months. At 24 months, the PLLA mesh implant maintained the internal space and the formed adipose tissue as well as that at 12 months, while the PLLA capsule implant collapsed and accumulated a large number of macrophages around the PLLA.

The PLLA mesh implant containing CS is a desirable bioabsorbable implant replaced by autologous adipose tissue after implantation in vivo without additional GFs or cells. Furthermore, we think that the main indication of this implant is the reconstruction after breast-conserving surgery. We are trying to regenerate larger amounts of adipose tissue using multiple PLLA mesh implants with CS in a porcine model and to create an irradiation model using a rodent.

Keywords

adipose tissue; PLLA; collagen sponge

Abstract **#299**

APPLICATION OF A SEA URCHIN-DERIVED COLLAGEN SKIN SUBSTITUTE FOR THE TREATMENT OF FULL-THICKNESS WOUNDS: A PRELIMINARY STUDY

Luca Melotti[P][1], Anna Perazzi[2], Tiziana Martinello[3], Ilaria Iacopetti[2], Michela Sugni[4,5,6], Marco Patruno[1]

[1] *Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (PD), Italy*

[2] *Department of Animal Medicine, Production and Health, University of Padova, Legnaro (PD), Italy*

[3] *Department of Veterinary Medicine, University of Bari, Valenzano (BA), Italy*

[4] *Department of Environmental Science and Policy, University of Milan, Milan, Italy*

[5] *Center for Complexity and Biosystems, Department of Physics, University of Milan, Milan, Italy*

[6] *GAIA 2050 Center, Department of Environmental Science and Policy, University of Milan, Milan, Italy*

Corresponding author's email: luca.melotti@unipd.it

Skin wounds are a challenging problem in the medical field and an economical burden. Skin substitutes (SS) are promising choices for wound healing since they mimic skin architecture and supports its regeneration. Here, we present a preliminary study about the application of a sea urchin-derived collagen-based SS [1, 2] in a second intention wound healing model. Two wounds were surgically created on the back of three sheep: one wound was treated with the SS and one was left untreated. The wound healing process was assessed with clinical observations and histological plus molecular analysis at 7, 14, 21 and 42 days.

The SS led to a better re-epithelialization than the placebo while no differences in wound contraction were observed. Histologically, treated wounds showed skin appendages already at day 14 and a higher cellular proliferation (Ki67+) with a faster resolution of the granulation tissue (GT) respect than the placebo. These results were further corroborated by gene expression levels of Collagen type I and III at 14 and 21 days. Concomitantly, a higher gene expression of VEGF (day 14) and a lower positivity for α-SMA+ cells (day 21 and 42) were observed in treated wounds.

The application of a sea urchin-derived SS led to a faster re-epithelialization, a better development of skin appendages and a properly maturation of GT. On the contrary, control wounds showed epidermal hyperplasia and dermal fibrosis. Overall, these preliminary findings suggest that this collagen-based SS possess beneficial properties, worthy of further investigations, for the treatment of full-thickness skin wounds.

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Abstract **#301**

OPTIMIZATION OF A PROTOCOL TO ISOLATE BOVINE ADIPOSE TISSUE-DERIVED MESENCHYMAL STROMAL CELLS

Emma Heyman[P][1], Bert Devriendt[1], Catharina De Schauwer[1]

[1] *Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium*

Corresponding author's email: emma.heyman@UGent.be

A wide diversity of procedures to isolate mesenchymal stromal cells (MSCs) from adipose tissue (AT) has been described in different species, including cattle. Although a high number of MSCs is often required for various tissue engineering approaches, isolation efficiency is rarely evaluated. To this end, diverse enzymatic digestion methods and one culture explant method were compared using subcutaneous AT samples of adult cows. Within the enzymatic digestion methods, different enzymes (collagenase I, collagenase I + trypsin, liberase, collagenase IV and MMP-12), enzymatic concentrations (0.25%, 0.1%, 0.04% and 400 ng/mL) and incubation times (3h, 6h, overnight and 24h) were evaluated. Plastic adherent cells were observed using most isolation methods on subcutaneous AT samples of 5 adult cows except when cells were isolated with: 0.1% collagenase I for 24h, 0.1% collagenase I + 0.25% trypsin overnight, 0.1% collagenase I + 0.25% trypsin for 24h, 0.04% collagenase IV for 3h and 0.04% collagenase IV overnight. Cells were collected in at least 4 out of 5 isolation repeats for the following methods: 3u coll I 0.1%, 3u lib 0.1%, 3u lib 0.04%, 6u lib 0.1% and 6u lib 0.04%. Enzymatic treatment with 0.1% liberase for 6h resulted in the highest average cell concentration (2,48 x 106 cells/mL) and cell viability (98,84%) when compared to the other methods. These cells were subsequently immunophenotyped and differentiated towards the osteogenic, chondrogenic and adipogenic lineage to confirm their MSC identity. In conclusion, the optimized protocol ensures the isolation of a high bovine MSC yield from subcutaneous AT.

Abstract **#302**

SELF-ASSEMBLY OF CHONDROGENIC SPHEROIDS FROM CELL-MONOLAYERS VIA GROWTH SURFACE SUBDIVISION

Marian Fürsatz$_P$[1,2], Peter Gerges[1,3], Susanne Wolbank[1,4], Sylvia Nürnberger[2,1,4]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology-AUVA research center, Vienna, Austria*

[2] *Department of Orthopaedics and Trauma-Surgery, Division of Trauma-Surgery, Medical University of Vienna, Vienna, Austria*

[3] *Institute of Applied Physics, Vienna University of Technology, Vienna, Austria*

[4] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

Corresponding author's email: marian.fuersatz@trauma.lbg.ac.at

Spheroid cultures are frequently used in vitro models and gain importance in clinics for regenerative applications. Drawbacks of current systems, especially for chondrogenic differentiation, are high reagent/time consumption and unavailability of early read-out parameters. Herein we present a system for self-assembly of chondrogenic spheroids from monolayers facilitated by growth surface subdivision using CO2-laser engraving (grid plates). Human telomerase reverse transcriptase immortalized adipose-derived stem cells (ASC/TERT1) mono- or co-cultures with primary human articular chondrocytes (hAC) were seeded on grid plates in low-dose differentiation media (1ng/ml TGF-β3/BMP-6), leading to self-assembly into spheroids. The diameter of generated spheroids was easily controlled by altering grid size, yielding approximately 340µm and 130µm for 3mm and 1mm grids respectively. Co-cultures formed spheroids significantly faster, reducing formation time from 3 weeks in mono-cultures to 1-2 weeks with increasing hAC content ($p<0.05$ and 0.01 for 1:1 and 1:4 ASC/TERT1:hAC ratio respectively). When comparing grid plate generated spheroids to standard pellets, similar differentiation capacity was observed. Internal structure however, showed differences in matrix distribution and cortex alignment. Grid spheroids were tested for applicability in regenerative settings by embedding into fibrin hydrogels, showing clear cellular outgrowth and collagen type 2 matrix deposition into the hydrogel, especially in co-cultures. First data also shows clear influence of pro- (IL-1β) and anti-inflammatory (dexamethasone) compounds on formation kinetics, making it a promising early read-out parameter candidate. In conclusion the herein presented system allows for efficient and reliable generation of spheroids via self-assembly from monolayer, showing promising results for in vitro and clinical applications.

Keywords

Autonomous spheroid formation; Chondrogenic differentiation; Compartmentation

Abstract **#303**

SURFACE ENGINEERING AND CELL ENCAPSULATION OF MIN-6 CELLS USING HYALURONIC ACID FOR THE TREATMENT OF DIABETES

Fernanda Zamboni[1,2], Ibrahim Fatih Cengiz[3], Cristiana Rodrigues Carvalho[3], Raquel Maia[3], Rui L. Reis[3], Joaquim Miguel Oliveira[3], Maurice N. Collins[1,2]

[1] Bernal Institute, School of Science & Engineering, University of Limerick, Limerick, Ireland

[2] Health Research Institute, University of Limerick, Limerick, Ireland

[3] 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães, Portugal

Corresponding author's email: fernanda.zamboni@ul.ie

Novel approaches to treat diabetes can include cell encapsulation and transplantation in order to mitigate graft rejection and restore physiological insulin secretion and normoglycemia [1]. In this work, a surface engineering approach was used to modify the plasma membrane of MIN-6 cells using a Mal-PEG-Lipid. The Mal-PEG-Lipid provides an anchorage site to the multiple hyaluronic acid derivate (HA-SH and HA-PD) coatings. The in vitro cell viability analysis for surface engineered MIN-6 cells showed that different Mal-PEG-Lipid concentrations (50, 250, and 500 µL per 1x106 cells) do not decrease cell viability as compared to control (approximate 90%). The incubation period for the cell membrane modification also shows no significant difference in cell viability after 30 minutes or 1 hour of incubation. After tagging Mal-PEG-Lipid with fluorescein, cells were incubated with the aforementioned concentrations, showing that cell fluorescence is time and concentration-dependent, with 500 µL Mal-PEG-Lipid per 1x106 cells incubated for 1 hour recording the highest fluorescence intensity and uptake. In conclusion, this novel encapsulation method is not harmful to pancreatic beta cells and has promising use in transplantation to treat diabetes.

Keywords

Surface engineering; Cell encapsulation; Conformal coating

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Abstract **#304**

MAGNETIC NANOPARTICLE MEDIATED ACTIVATION OF THE ACTIVIN A RECEPTOR FOR TENOGENIC DIFFERENTIATION

Lucrezia Righelli$_P$[1], Hareklea Markides[1], Ana Gonçalves[2], Manuela Gomes[2], Alicia El Haj[1]

[1] *University of Birmingham, UK*

[2] *University of Minho, Portugal*

Corresponding author's email: LXR866@student.bham.ac.uk

Tendons display poor intrinsic healing properties and are difficult to treat[1]. In this study, we investigate whether there are specific mechano-active regions of the Activin A receptor which can influence the tenogenic differentiation of mesenchymal stromal cells (MSCs) using a magnetic bioreactor (MICA Biosystems Ltd)[2,3]. The role of oriented 3D collagen models[4,5,6] combined with Activin A activation is further explored.

Magnetic nanoparticles (MNPs) functionalized with 3 anti-Activin Receptor IIA antibodies targeting different regions of the receptor, were screened by FACS and immunofluorescence. MNPs-labelled cells were then stimulated with MICA and the activation of the Smad2/3 pathway assessed by ELISA. MNPs-labelled cells were then differentiated in tenogenic media for 21 days with 1h daily MICA stimulation in 2D and 3D oriented collagen models. Tenogenesis was assessed by histological staining and IHC. 3D oriented fibres collagen models were achieved by labelling MSCs with MNPs and setting the gels under a constant magnetic field. The fibres alignment was assessed by polarized and confocal microscopy.

Screening of multiple antibodies revealed binding to MSCs. Cells treated with tenogenic media in MICA-stimulated conditions showed an increase in collagen deposition and tenomodulin and scleraxis expression in 2D and 3D. Increased phosphorylation of the Smad2/3 complex was detected in MICA-activated groups as determined by ELISA. The polarised and confocal microscopy showed significant orientation of collagen fibres in gels set under magnetic conditions. In conclusion, the MICA remote activation of Activin A receptor using tagged MNPs enhances the tenogenic response in MSCs both in 2D and 3D models.

Keywords

Magnetic Nanoparticles; Magnetic Bioreactor; 3D Collagen Gels

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Abstract **#305**

CRISPR ACTIVATION OF LONG NON-CODING RNA DANCR PROMOTES BONE REGENERATION BY INHIBITING MIR-203A AND MIR-214

Thi Kieu Nuong Nguyen[P][1], Anh Vu Truong[1], Mu-Nung Hsu[1], Ya-Hui Lin[1], Yi-Hsiu Wu[1], Yu-Chen Hu[1]
[1] *Department of Chemical Engineering, National Tsing Hua University, Hsinchu 300, Taiwan*
Corresponding author's email: nuongnguyenkn@gmail.com

Adipose-derived mesenchymal stem cells (ASCs) have the potential of multi-lineage differentiation and differentiate into either chondroblasts or osteoblasts, leading to bone formation and bone regeneration. Long-non coding RNAs (lncRNAs) play crucial roles for gene transcription and mRNA expression in regulating various cellular processes such as cell differentiation, cell development, and cell proliferation. The previous studies have confirmed that lncRNA DANCR (DANCR) upregulated chondrogenesis in mesenchymal stem cells. However, the role of DANCR in controlling chondrogenic differentiation of stem cells and its molecular mechanism in chondrogenesis remains unknown. In this study, we further aimed to harness CRISPR activation (CRISPRa), which exploits single guide RNA (sgRNA) and nuclease-deficient Cas9 (dCas9) protein. dCas9 can be fused with a tripartite transcription activator domain (dCas9-VPR) that can stimulate endogenous DANCR expression. To this end, we constructed different baculovirus vectors to express CRISPRa VPR system fused with dCas9 derived from different organisms. We observed that the CRISPR-SadCas9-VPR activator significantly upregulated DANCR expression and stimulated the expression of chondrogenic differentiation markers such as Sox9, Col2, Acan. Moreover, implantation of the engineered rASC into the critical size defects at the calvaria of SD rats significantly enhanced the calvarial bone healing. Furthermore, we identified that DANCR promoted the rASC chondrogenic differentiation through inhibition of miR-203a and miR-214. Taken together, our results indicated that DANCR activation by SadCas9-VPR-based CRISPRa provides a novel therapeutic approach for regenerative medicine to improve bone healing.

Keywords
CRISPR; lncRNA; calvarial bone regeneration

Abstract **#306**

MESENCHYMAL STEM/STROMAL CELLS (MSCS) ARE DEVELOPMENTALLY PROGRAMMED – IMPLICATIONS FOR TISSUE REPAIR APPLICATIONS

Cristina Esteves[P][1], Emma Weatherall[1], Viktorija Avilkina[1], Yennifer Cortes-Araya[1], Susan Dan-Jumbo[1], Claire Stenhouse[1], Francesc Xavier Donadeu[1]

[1] *The Roslin Institute, University of Edinburgh*

Corresponding author's email: Cristina.Esteves@roslin.ed.ac.uk

Consistency in clinical outcomes is key to the success of therapeutic Mesenchymal Stem/Stromal cells (MSCs) in regenerative medicine. However the properties of MSC preparations can vary significantly with different factors. We studied the effects of developmental programming associated with intrauterine growth restriction (IUGR) on MSC properties. IUGR affects 10-40% of newborns worldwide, results from inadequate uterine capacity and placental insufficiency of multifactorial origin, and is linked to increased risk of metabolic (e.g. diabetes) and other diseases later during life.

Here, we compared the properties of adipose-derived MSCs from IUGR (IUGR-MSCs) and normal (Normal-MSCs) newborn pig littermates. IUGR occurs spontaneously in multiparous species such as the pig where it shows features that recapitulate human IUGR.

Both MSC types grew clonally and expressed typical MSC markers (CD105, CD90, CD44) at similar levels. Importantly, tri-lineage differentiation capacity was significantly altered by IUGR. IUGR-MSCs had higher adipogenic capacity than Normal-MSCs as evidenced by significantly higher adipocyte content and expression of the adipogenic transcripts, PPARg and FABP4 after 12 days of differentiation. A similar trend was observed for fibrogenesis, where IUGR-MSCs expressed significantly higher levels of C1A1 than Normal-MSCs. In contrast, chondrogenic and osteogenic potential were decreased in IUGR MSCs as shown by a smaller chondrocyte pellet and osteocyte staining, and lower expression of the transcription factors SOX9 and RUNX2 implicated in chondrogenic and osteogenic differentiations, respectively.

In conclusion, the regenerative potential of MSCs appears to be determined prenatally and this should be taken into account when selecting cell donors in regenerative therapy programmes.

Abstract **#307**

AN INJECTABLE CEO2 NANOENZYME@PEG HYDROGEL FOR THE TREATMENT OF ACHILLES TENDINITIS AND TENDON DEGENERATION

Xingquan Xu[P][1], Sheng Zhao[2], Rongliang Wang[1], Rui Wu[1], Qing Jiang[1], Dongquan Shi[1]

[1] *Department of Sports Medicine and Adult Reconstructive Surgery, Drum Tower Hospital, School of Medicine, Nanjing University*

[2] *College of Engineering and Applied Sciences, Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University*

Corresponding author's email: xuxingquan12345@163.com

Background：

Achilles tendinitis is a common disease, which is characterized by pain, swelling, and stiffness of Achilles tendon. The purpose of this study was to investigate whether the injectable CeO2 nanoenzyme@PEG hydrogel could alleviate inflammation and degeneration of Achilles tendon.

Methods：

Thirty male SD rats were received collagenase injections into their bilateral Achilles tendons (collagenase-induced tendinopathy model). The rats were randomly divided into five groups: CeO2 NPS, CeO2 NPS+hydrogel, hydrogel, diclofenac, and control group. After four weeks' intratendinous injection, the Achilles tendons were harvested and subjected to histological analyses (HE and Masson staining). Tertiary butyl peroxide of hydrogen (TBHO) was used to treat primary tenocytes to mimic oxidative stress-mediated injury. And, inflammation levels and Nrf2 expression was evaluated after CeO2 NPS treatment. The inflammation levels were also evaluated after the cells were transfected with Nrf2 small interfering RNA.

Results:

Compared to the control and diclofenac group, CeO2 NPS treatment resulted in better healing of injured tendon with improved histological outcomes. Combination PEG hydrogels with CeO2 NPS significantly enhanced the effects of CeO2 NPS treatment along. QPCR and Western showed that IL-1β and IL-6 activity were enhanced in tenocytes after TBHO treatment. While, CeO2 NPS could induce Nrf2 activity and suppress IL-1β and IL-6 expression.

Conclusion

CeO2 NPS could enhance healing of injured Achilles tendon by NRF2 mediated inflammation suppression. CeO2 NPS PEG hydrogels composite has the potential to be clinically used to treat tendinitis.

Abstract **#308**

DETERMINATION OF MINIMAL METABOLIC REQUIREMENTS OF STEM CELL SURVIVAL USING A GRADIENT GENERATING DYNAMIC MICROFLUIDIC CELL CULTURE PLATFORM

Melvin Gurian$_P$[1], Yvonne Schreurs[1], Liliana Moreira Teixeira[1], Jeroen Leijten[1]

[1] *Department of Developmental BioEngineering, University of Twente, The Netherlands*

Corresponding author's email: m.gurian@utwente.nl

Metabolites are essential for the survival, function, and fate of cells(1,2). However, current knowledge is typically based on static culture systems in which cells are exposed to an initially chemically controlled medium, which rapidly decreases in its nutrient content over time(3). Consequently, this has limited our understanding of the critical metabolite concentrations that determine cell survival and function. We here report on the development and use of a dynamic microfluidic-based cell culture platform for the determination of minimal metabolic requirements for the survival and function of mesenchymal stem cells. The microfluidic devices was contained a gradient generator, offering long-term stable and linear control over the media used for the perfusion of a parallelized array of cell culture chambers. Subsequently, approximately 6000 cells were seeded per cell culture chamber and exposed to a range of metabolite concentrations. We confirmed that the dynamic media flow was sufficiently fast to maintain constant metabolite concentrations, while keeping the hydrodynamic shear sufficiently low (<0.3 dyn/cm2)(4) to avoid causing effects on cell behavior. Cells were then cultured over a maximal period of seven days to quantitatively determine the minimal concentrations to sustain cell survival as well as function for a panel of relevant metabolites in a time-resolved manner. This approach allowed the determination of the relative importance of individual metabolites on the survival of mesenchymal stem cells, which offers novel fundamental insights what is required to avoid starvation-induced cell loss of implanted cells and prevascular tissues.

Keywords

microfluidics; metabolites; cell survival

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Abstract **#309**

IMPROVING THE FUNCTIONALITY OF BIOFABRICATED MYO-SUBSTITUTES USING A TAILORED ELECTRO-MECHANICAL BIOREACTOR

Marco Costantini[P][1], Nehar Celikkın[1], Dario Presutti[1], Cesare Gargioli[2]

[1] *Institute of Physical Chemistry - Polish Academy of Sciences*

[2] *Department of Biology, Rome University Tor Vergata, Rome, Italy*

Corresponding author's email: mcostantini@ichf.edu.pl

The key role of skeletal muscle tissue in the human body is undoubted being the controller of several vital functions including respiration and all voluntary locomotion activities. However, its regenerative capability is limited and, in the case of a significant tissue loss, there is often no medical treatment available to restore or repair the lost tissue. Therefore, it is of the utmost importance the development of new biotechnological approaches that would eventually enable the manufacturing in vitro of functional, spare tissue part ready to be implanted in the patient. In this context, we propose a biofabrication approach combined with a custom electro-mechanical bioreactor to rapidly fabricate myo-substitutes that exhibit a bio-mimetic histoarchitecture and a functionality close to native tissue.

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Abstract **#310**

FLUIDISED-BED BIOREACTOR FOR REGULATORY T CELLS EXPANSION; THE EARLY PHASE OF DESIGN AND OPTIMISATION

Piyanan Boonprasirt$_P$[1], Marianne J Ellis[1]

[1] *Centre for Integrated Bioprocessing, Department of Chemical Engineering, University of Bath, Bath, BA2 7AY, UK*

Corresponding author's email: pb676@bath.ac.uk

The challenge after organ transplantation is graft rejection, which can be prevented by using immunosuppressants. However, there are side effects after long-term treatment with the medications. Recently, cell-based therapy using Regulatory T cells (Tregs) to modulate the immune system has been extensively studied to replace conventional treatment. As a billion cells are needed for the treatment, cell culture device for large-scale production is required. Fluidised-beds reactors (FBR) have been developed for several chemical systems. However, only a few numbers of studies are presented in the area of mammalian cell cultures. The advantages of using a fluidised bed bioreactor (FBB) for cell culture is the system provides a realtively high cell density and well-mixing condition with low shear force [1, 2]. This study aims to design and optimized cell culture conditions in FBB for ex vivo Tregs expansion.

This study is the early phase of FBB design using the non-adhere cell line HL60 as a cell model. The first stage of FBB design was to determine two important factors; minimum fluidising velocity (umf) and terminal velocity (ut). The velocity for cell culture in FBB was selected between these two values where the cells were proliferated without losing them from the bioreactor. Then the parameters that affect mass (nutrient, oxygen, toxic metabolites) transfer and shear stress were identified. In this study, three parameters include velocity, column diameter, initial cell number were studied using Design of Experiments, with the aim of selecting operating regions ready for ex vivo Tregs expansion studies.

Keywords

Regulatory T Cells; Fluidised-bed; Bioreactor Design

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Abstract **#311**

SMALL EXTRACELLULAR VESICLE-MIR-381 DERIVED FROM KARTOGENIN-PRECONDITIONED MESENCHYMAL STEM CELLS PROMOTE CHONDROGENESIS

Xiaomin He[P][1], Hui Jing[1], Jinghao Zheng[1]

[1] *Shanghai Children's Medical Center*

Corresponding author's email: mrxmhe@163.com

Small extracellular vesicles (sEVs) derived from mesenchymal stem cells have been shown to possess potent regenerative potential. In this research, we evaluated the chondrogenic effect of sEVs derived from Kartogenin-preconditioned human umbilical cord mesenchymal stem cells (hUCMSCs). sEVs were isolated from the supernatants of KGN-preconditioned hUCMSCs (KGN-sEV) by gradient ultra-centrifugation, and internalized by native hUCMSCs, thereby inducing the chondrogenic differentiation. The underlying mechanism of KGN-sEV induced chondrogenesis was explored by high-throughput sequencing and verified by transfection with the corresponding mimic and inhibitor. Sequencing identified the unique enrichment of a set of miRNAs in KGN-sEV, compared with sEVs derived from unpreconditioned cells (un-sEV). Overexpression/inhibition in vitro and in vivo demonstrated that this chondrogenic potential was primarily attributed to miR-381-3p, one of the most abundant miRNAs in KGN-sEV. Dual-luciferase reporter assays showed that miR-381-3p promoted chondrogenesis, through directly suppressing TAOK1 by targeting its 3’ untranslated region and thereby suppressing the Hippo signaling pathway. Collectively, our results highlight the regenerative potential of KGN-sEV to induce chondrogenic differentiation of MSCs, which is achieved mainly by delivering sEV-miR-381-3p, which targets TAOK1.

Keywords

small extracellular vesicles; mesenchymal stem cells; chondrogenesis

Abstract **#312**

HGF/HEPARIN-IMMOBILIZED DECELLULARISED LIVER MATRIX FOR ACUTE CHEMICALS-INDUCED LIVER INJURY REGENERATION

Yi Cheng Hsieh[P][1]

[1] *Department of Biomechatronics Engineering, NTU*

Corresponding author's email: r09631037@ntu.edu.tw

Liver cirrhosis has long been considered as a common death in the world. However, the existing methods remain some challenge, such as alleviating or ceasing the symptom of the liver cirrhosis instead of regenerating hepatocytes. Notably, many research mentioned that liver fibrosis is reversible, thus once we treat liver fibrosis properly in time, it will not lead to end stage (liver cirrhosis) or even be cured.

Therefore, for the first time, a HGF/heparin-immobilized decellularised liver matrix (HGF/heparin-DLM) was developed for hepatocytes regeneration in acute chemicals-induced liver injury, which the chemicals we chose such as CHCl3 were proved to induce liver fibrosis. The amounts of immobilized heparin on DLM film was approximately 180 μg/cm2 when the initial concentration of heparin was 1 mg/mL. After three days of culture, the relative cell viability and albumin synthesis of the hepatocytes on HGF/heparin-DLM was 20-30% and 20% superior than that on normal dish, respectively.

From the result of CHCl3-induced model, the lactate dehydrogenase activity of injured hepatocytes on HGF/heparin-DLM showed lower cytotoxicity than normal one, meanwhile the relative viability of those on the film were higher, both of which showed the ability of regeneration. Last but not least, the albumin synthesis of injured hepatocytes on HGF/heparin-DLM can recover to almost 70%-80% the same level as non-toxic hepatocytes cultured on normal dish.

In summary, the HGF/heparin-DLM has positive effect on culturing hepatocytes and regenerating damaged ones. Furthermore, the HGF/heparin-DLM can be utilized for future application of liver tissue.

Keywords

decellularised liver matrix ; hepatocyte growth factor; acute chemicals-induced liver injury

Abstract **#313**

LIQUEFIED MICROCAPSULES COMPARTMENTALIZING MACROPHAGES AND UMBILICAL CORD-DERIVED CELLS FOR BONE TISSUE ENGINEERING

Sara Nadine[1], Clara R. Correia[1], João F. Mano[1]
[1] *CICECO - Aveiro Institute of Materials, University of Aveiro, Portugal*
Corresponding author's email: saranadine@ua.pt

The immune system plays an indispensable regulatory role in the progress of bone regeneration, with special focuses on macrophage activity.[1] Inspired on the native dynamics between immune and skeletal systems, we propose an in vitro bioengineering system of a biomimetic bone niche that comprises liquefied and multilayered microcapsules co-encapsulating macrophages, mesenchymal stromal cells (MSCs), and endothelial cells (ECs). Additionally, surface-functionalized poly(ε-caprolactone) microparticles are loaded into the liquefied core to provide cell adhesion sites. To better mimic the dynamic environment of native tissues, microcapsules are dynamically cultured in spinner flasks.[2] Afterwards, liquified microcapsules are cultured up to 21 days in basal or osteogenic differentiation media. Microcapsules without macrophages are used as control. The main goal is to promote a well-orchestrated cell-to-cell interaction enabling the evaluation of the bioperformance of macrophages toward bone tissue repair. Results show that cellular viability remained uncompromised after 21 days of culture. Notably, the osteogenic osteocalcin marker was only found in microcapsules encapsulating macrophages. Mineralization evidence can be observed in all conditions, with a Ca/P ratio similar to the native hydroxyapatite. Additionally, microcapsules encapsulating macrophages released significantly more osteoprotegerin, osteopontin, and vascular endothelial growth factor comparing with the MSCs+ECs condition. Accordingly, we intend to use the proposed system as hybrid devices implantable by minimally invasive procedures for bone tissue engineering applications.

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Abstract **#314**

COATINGS OF LOW ENDOTOXIN GELATIN IMPROVE STEM CELL SEEDING EFFICIENCY AND PROLIFERATION ON 3D PRINTED DISCS IN VITRO

Jos Olijve[P][1], Barbara Vanhoecke[1]

[1] *Rousselot - Gelatin*

Corresponding author's email: JOS.OLIJVE@ROUSSELOT.COM

INTRODUCTION

Endotoxins are contaminants which can trigger immune responses in vivo. The FDA has imposed restrictions on endotoxin content in medical device for in-body use. However, in cell-based in vitro experiments there is limited attention for the side-effects of endotoxins even when endotoxin-sensitive cells are used. We examined stem cell seeding efficiency/proliferation on coatings of different materials containing different endotoxin levels.

METHODS

3D printed and plasma activated acrylic discs were coated with gelatins of different purities and molecular weights (Rousselot and Sigma) and with 'standard' materials fibronectin and laminin (Sigma). Endotoxin levels were evaluated using the EndoZyme assay. 1µg/ml of the materials were added to the discs and allowed to adhere for 90min at RT. hTERT adipose derived MSCs were seeded at a density of 5,700 cells/cm² onto the coated discs and cultured in AdipoUp medium at 37°C. Lactate release in the medium was evaluated over 7 days as read-out for cell proliferation.

RESULTS

hTERT MCS cells grew better on gelatin coatings compared to laminin and fibronectin. More lactate was released when purified gelatins were used compared with unpurified gelatins suggesting a negative effect of endotoxins on cell growth. Best results were obtained when purified, low molecular weight gelatin was used indicating that molecular weight of the gelatin coating material play a role in cell adhesion and proliferation.

CONCLUSIONS

The results from this study show that endotoxin might affect stem cell behavior. The results indicate that it is important to work with purified materials in-vitro also to enhance clinical translation.

Abstract **#315**

HIGHLY POROUS, ELASTIC, INJECTABLE AND BIODEGRADABLE BIPHASIC CALCIUM PHOSPHATE CERAMIC SPONGE FOR BONE REGENERATION

Yonggang Zhang[1], Pamela Habibovic[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Instructive Biomaterials Engineering, Maastricht University*

Corresponding author's email: y.zhang@maastrichtuniversity.nl

In the past decades, extensive research efforts have been expended in the fields of materials science and scaffolding technologies to fabricate synthetic bone graft substitutes. Among various types of biomaterials, biodegradable porous calcium phosphate (CaP) ceramics, such as beta-tricalcium phosphate (β-TCP) and biphasic calcium phosphate (BCP, mixture of hydroxyapatite (HA) and β-TCP) are potentially interesting bone graft substitutes, owing to their excellent osteoconductivity and, depending on their physico-chemical properties, even the ability to induce de novo bone formation [1, 2]. However, the intrinsic brittleness of CaP ceramics leads to poor processability, stability and handling properties, and their clinical application is limited to non load-bearing- applications. Herein, we developed a BCP ceramic sponge that is fully inorganic but elastic, highly porous and biodegradable, by using anisotropic calcium phosphates crystals as building blocks. The BCP sponge comprises a self-supporting highly porous network of seamlessly interwoven HA nanowires and β-TCP nanofibers. This BCP ceramic sponge exhibits several interesting properties including ultrahigh porosity (>99% free volume), interconnected porous structure and mechanical stability which are crucial factors to provide a suitable environment for cell attachment, migration, proliferation, mass transport and tissue deposition [3]. Besides, this BCP ceramic sponge is surprisingly elastic, endowing it very good stability and handling properties. Moreover, the BCP ceramic sponge is injectable and shows exciting shape-memory behavior. Taken together, the BCP sponge developed here presents an appealing alternative to current brittle ceramics for bone regeneration applications.

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Keywords

Biomaterials; Bone regeneration; Calcium phosphate

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Abstract **#316**

SECRETOME FROM MESENCHYMAL STEM CELLS AS A PROTECTIVE AGENT AGAINST THE NEURODEGENERATIVE ACTION OF ALPHA-SYNUCLEIN – A STUDY ON CAENORHABDITIS ELEGANS MODELS OF PARKINSON'S DISEASE

Cláudia Marques$_P$[1,2], Joana Pereira-Sousa[1,2], Sofia Serra[1,2], Rui Sousa[3], Andreia Teixeira-Castro[1,2], António Salgado[1,2]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Portugal*

[2] *ICVS/3B's – PT Government Associate Laboratory, Portugal*

[3] *Stemmatters, Biotecnologia e Medicina Regenerativa S.A., Portugal*

Corresponding author's email: id6511@alunos.uminho.pt

This study has investigated the capacity of secretome from bone marrow-derived mesenchymal stem cells (BM-MSCs) to prevent dopaminergic neuron degeneration caused by overexpression of alpha-synuclein in two Caenorhabditis elegans models of Parkinson's Disease (PD).

Secretome prepared in two different concentrations was tested in a more predictive model of PD that overexpresses alpha-synuclein in the dopaminergic neurons. These animals display progressive dopaminergic neurodegeneration that is age dependent. Moreover, given the transparency of the animal and the expression of green fluorescent protein in dopaminergic neurons, is possible to observe the presence/absence of these cells. Animals were treated with secretome since the embryo stage for 7 days. At the seventh day the number of intact dopaminergic neurons was scored. One of the prepared secretome was also tested in a C. elegans strain that overexpresses alpha-synuclein in the body wall muscle cells. Given the transparent body of the animal and the expression of alpha-synuclein fused to yellow fluorescent protein, is possible to observe alpha-synuclein inclusions throughout the entire body of the animal. After 4 days submitted to treatment with secretome since the embryo stage, the number of alpha-synuclein inclusions in the head region was determined. Animals treated with secretome 1x concentrated showed reduced dopaminergic neurodegeneration. Furthermore, secretome displayed a mild effect in the reduction of the number of alpha-synuclein inclusions.

These results show that secretome from BM-MSCs plays a neuroprotective role against the action of alpha-synuclein, a hallmark of Parkinson's Disease, indicating its potential as a new therapeutic solution for future regenerative medicine strategies.

Abstract **#317**

BIO-FABRICATION OF MINERALIZED HUMAN AMNIOTIC MEMBRANE AS A BIOMIMETIC HARD TISSUE ENGINEERING SCAFFOLDS

Leila Sabouri[P][1], Mahmoud Azami[2], Mehdi Moghtadaei[1,3]

[1] *Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*

[2] *Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

[3] *Department of Orthopaedic Surgery, Rasoul-e-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.*

Corresponding author's email: sabourileila@yahoo.com

"Simple but useful" is the title that can be given to the amniotic membrane after years of research in the field of tissue repair and regenerative medicine. The success of amniotic membrane process in restoring various tissue has been reported. Amniotic membrane is one of the few materials used in both soft and hard tissue engineering. However, some its mechanical and biological properties such as rapid biodegradability are an obstacle to human amniotic membrane widespread and effective use in hard tissue engineering. In the present study, human amniotic membrane decellularized matrix was developed as a substrate and then mineralization was performed by a biomimetic method. In this way, calcium and phosphate ions were deposited on the surface and inside of decellularized human amniotic membrane and strengthened its properties for hard tissue engineering application. Real-time PCR results showed a high expression of osteogenesis related genes from adipose-derived mesenchymal stem cells (ADMSCs) cultured on the surface of mineralized human amniotic membrane. Further in-vivo experiments were conducted using mineralized human amniotic membrane pre-seeded with ADMSCs and in a critical-size rat calvarial defect model. Histopathological results confirmed that the mineralized human amniotic membrane with ADMSCs sample has great potential in bone regeneration.

Abstract **#319**

COMBINATORIAL EFFECTS OF HYALURONIC ACID CONCENTRATION AND MECHANICAL MODULUS ON HUMAN NEURAL PROGENITOR CELLS IN 3D CULTURE

Stephanie Seidlits[1], Savannah Shumock[1], Laila Rad[1], Rebecca Bierman[P][1]
[1] *University of California Los Angeles, Department of Bioengineering*
Corresponding author's email: rbierman1@ucla.edu

Neural progenitor cells (NPCs) in the adult central nervous system (CNS) have the innate capacity to regenerate specialized cells lost due to injury or disease. However, their response is often not sufficient to restore function. Biomaterials are promising tools to improve NPC-mediated repair as they can provide a pro-regenerative microenvironment and a carrier for transplantation of exogenous NPCs. Previously, we reported that hydrogels based on hyaluronic acid (HA), a native component of CNS matrix, support 3D culture of human NPCs for at least 70 days and, compared to 2D controls, bias differentiation towards neurons and oligodendrocytes (1). Culture in HA-based hydrogels with mechanical moduli on the order of those in native CNS tissue (<1 kPa) has also been reported to improve viability and affect differentiation (1,2). Here, we used a biomaterial platform to investigate the combined effects of hyaluronic acid (HA) content and matrix mechanics on the regenerative potential of human NPCs. A full factorial experimental design was used where HA was varied from 0.1–1 wt% and shear storage modulus from 200–800 Pa. Over 1 week in culture, NPC proliferation rate generally increased with increasing HA concentration and decreasing modulus. Of the 9 formulations evaluated, 200 Pa hydrogels with 0.5 wt% HA best promoted NPC differentiation towards neuronal and oligodendrocyte lineages after 2 weeks of culture. As previously reported (2), GFAP expression was not observed in 3D cultures. Results will inform future designs of NPC-instructive, hydrogel scaffolds for CNS regeneration.

Keywords
hyaluronic acid; neural progenitor cells; mechanical properties

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Abstract **#320**

NEW STRATEGIES IN ENGINEERING POLYANILINE ELECTROSPUN NANOFIBERS FOR NEURAL TISSUE APPLICATIONS

Fábio F. F. Garrudo[P1,2,3], Paiyz M. Esmat[1], Carlos A.V. Rodrigues[2], Diogo E. S. Nogueira[2], João C. F. Silva[2,1], Ranodhi N. Udangawa Keating[1], Flávio A. Ferreira[2], Caitlyn A. Chapman[1], Pauline R. Hoffman[1], Joaquim M. S. Cabral[2], Jorge Morgado[3], Frederico C. Ferreira[2], Robert J. Linhardt[1]

[1] Department of Chemical and Biological Engineering, Biology and Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary studies, Rensselaer Polytechnic Institute, 110 8th street, 12180 Troy, NY, USA

[2] Department of Bioengineering, Institute for Bioengineering and Bioscience, Instituto Superior Técnico, Universidade de Lisboa, Av Rovisco Pais, 1049-001 Lisboa, Portugal

[3] Department of Bioengineering, Universidade de Lisboa and Instituto de Telecomunicações, Av Rovisco Pais, 1049-001 Lisboa, Portugal

Corresponding author's email: fabiogarrudo@gmail.com

We report the development of a biodegradable coaxial electrospun nanofiber scaffolds for the electrical stimulation of NSCs. This coaxial fiber consists of an external layer of polycaprolactone(PCL) and polyaniline(PANI) and a core layer composed of poly(glycerol sebacate)(PGS). The construction of such platform required the accomplishment of 3 sequential research steps:

1. Optimization of PANI to PCL ratio;
2. Electroconductivity enhancement through solvent system modulation;
3. Production of an electrospun conductive coaxial fiber.

Electroconductive fibers can be obtained from different PCL-PANI ratios 88:12,91:9,93:7,94:6 and 95:5, with conductivities ranging from 0.014 to 0.077Scm-1. All combinations tested are biocompatible for NSC culture. Electroconductivity was dependent on environmental humidity.

The solvent system for PCL-PANI fibers was optimized using TFE and hexafluoropropanol (HFP). The best combination was used to produce electrospun fibers with higher electroconductivity (0.2Scm-1) at a higher humidity (50%). This effect was attributed to the pseudo-doping of PANI by HFP. The AC electrical stimulation of NSCs was performed and differentiation was enhanced.

Finally, coaxial PCL-PANI/PGS fibers were produced (951±465nm of diameter, electroconductivity of 0.063±0.029Scm-1). The mechanical properties (ε=1.3MPa) and hydrophilicity (38º) were also favorable for NSC culture. iPSC differentiation towards the neural lineage was favored by the presence of the coaxial fibers as shown by the upregulation of DCX, NCAM, NEUN and GAD67 neural markers, without and with electrical stimulation.

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Abstract **#321**

NOVEL BIOMATERIAL-BASED PLATFORMS FOR THE ELECTRICAL STIMULATION OF STEM CELLS

Fábio F. F. Garrudo[P][1,2,3], Laura Sordini[2,3], João C. F. Silva[2,1], Giulia Fillipone[2], Flávio A. Ferreira[2], Eliana C. Pereira[2], Ranodhi N Udangawa Keating[1], Paiyz E. Mikael[1], Carlos A. V. Rodrigues[2], Joaquim M. S. Cabral[2], Robert J. Linhardt[1], Jorge Morgado[3], Frederico C. Ferreira[2]

[1] Department of Chemical and Biological Engineering, Biology and Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary studies, Rensselaer Polytechnic Institute, 110 8th street, 12180 Troy, NY, USA

[2] Department of Bioengineering, Institute for Bioengineering and Bioscience, Instituto Superior Técnico, Universidade de Lisboa, Av Rovisco Pais, 1049-001 Lisboa, Portugal

[3] Department of Bioengineering, Universidade de Lisboa, and Instituto de Telecomunicações, Av Rovisco Pais, 1049-001 Lisboa, Portugal

Corresponding author's email: fabiogarrudo@gmail.com

Electrical stimulation can have various beneficial effects on stem cells, including the promotion of their proliferation and differentiation profiles. This is essential for tissue engineering strategies, as the phenotype of the obtained cells is better suited to mimic that of the target tissue. They can be used as reliable in vitro models for disease study and cell transplantation.

The design of platforms suitable for electrical stimulation of stem cells requires a multidisciplinary approach. Through the manipulation of material's chemistry, tri-dimensionality and bioactive cues it is possible to direct cell colonization and create different cell domains that can mimic the in vivo complexity of tissues.

Our group has worked on different electrospun composites to create suitable electroconductive platforms for the electrical stimulation of stem cells, aiming at neuronal, cardiovascular, cartilage and bone tissue engineering applications. Some of the strategies include (a)optimization of poly(3,4-ethylenedioxythiophene)(PEDOT) composites, (b)doping of polybenzimidazole(PBI), (c)cross-linking of polyacrylonitrile(PAN), (d)optimization of polycaprolactone(PCL)–polyaniline(PANI) blend, and (e)production of electroconductive coaxial fibers.

Our results justify a combined approach of materials science with the glycobiology of the cultured cells for the development of biomimetic electroconductive platforms. We believe such strategy is essential to develop biomaterials capable of enhancing stem cell survival and differentiation. In the future, such strategies can be used to improve the tissue integration of transplanted cells.

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Abstract **#322**

BIOFABRICATION OF AN IN VITRO PRIMARY 3D OSTEOSARCOMA MODEL (3D-OSM)

Serena Duchi[P][1,2,3], Wiktor Zywicki[2,4], Thomas Chow[2,5], Stephanie Doyle[2,6], Cathal D O'Connell[2,6], Carmine Onofrillo[1,2,3], Peter F Choong[1,2,7], Claudia Di Bella[1,2,7]

[1] *University of Melbourne, Department of Surgery at St. Vincent's Hospital Melbourne (SVHM), Fitzroy 3065, VIC, Australia*

[2] *BioFab3D, Aikenhead Centre for Medical Discovery-SVHM, Fitzroy 3065, VIC, Australia*

[3] *ARC Centre of Excellence for Electromaterials Science, Intelligent Polymer Research Institute, Innovation Campus, University of Wollongong, NSW, Australia*

[4] *Department of Biomedical Engineering, University of Melbourne, Parkville, VIC, Australia*

[5] *Melbourne Medical School, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville 3052, VIC, Australia*

[6] *Discipline of Electrical and Biomedical Engineering, School of Engineering, RMIT University Melbourne, VIC, Australia*

[7] *Department of Orthopaedics, St Vincent's Hospital Melbourne (SVHM), 3065 Fitzroy, VIC, Australia*

Corresponding author's email: sduchi@unimelb.edu.au

Osteosarcoma (OS) is a highly aggressive primary bone tumour, which preferentially arises in the metaphysis of actively growing long bones. OS predominantly affects children and young adults, and it is characterized by synthesis of disorganized mineralized bone by the tumour cells (1). Unfortunately, current in vitro preclinical models such as monolayer cell culture, fail to recapitulate the tumour microenvironment and thus accurate neoplastic behaviour, ultimately hindering therapeutic discovery (2). The employment of three-dimensional OS models has the potential to better recreate the spatial, mechanical, and biological complexity of the tumour microenvironment, key elements crucial for understanding tumour prognosis and treatment effectiveness (3).

In this study, we propose an in vitro primary 3D OS Model (3D-OSM) comprising an inner core of OS cells embedded within biocompatible Gelatin Methacryloyl (GelMA), a surrounding shell of bone-mimicking scaffold (Polycaprolactone), and cultivated in a microfluidic system device. The core mimics the stromal fraction of OS, while the shell represents the bone niche where the tumour usually arises and growth. The model has been validated using OS cell lines and patient derived tumour cells (canine osteosarcoma) with metabolic assays to measure cell viability during time. Non-destructive computed tomography and confocal imaging was used to evaluate the extravasation of tumour cells toward the scaffold.

Our findings establish the conditions that ensure optimal survival and proliferation of OS tumour cells and validates our biofabrication approach. Further development of the 3D-OSM could revolutionize preclinical high throughput compound discovery, together with potential personalized medicine treatments.

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Abstract **#323**

ON-DEMAND RELEASE OF DOXORUBICIN BY NEAR INFRARED LIGHT-RESPONSIVE MATERIALS FOR PANCREATIC CANCER

Lilith Caballero Aguilar$_P$[1,2], Shaun Geitman[2,3], Kara Vine[4], Simon Moulton[5,6,7]

[1] *BioFab3D, St. Vincent's Hospital. Australia*

[2] *Swinburne University of Technology, Australia*

[3] *ARC Centrre for Electromaterials Sciences*

[4] *Illawarra Health and Medical Research Institute*

[5] *Swinburne University of Technology*

[6] *ARC Centre for Electromaterials Science*

[7] *BioFab3D, St. Vincent's Hospital, Australia*

Corresponding author's email: lcaballeroaguilar@swin.edu.au

Pancreatic ductal adenocarcinoma (PDAC) has the lowest survival of any solid tumour, with a cumulative five-year survival rate of 6.8% (1). Despite the development of new anticancer agents and immuno-oncology drugs, PDAC remains highly refractory to systemically delivered therapies. This is due in part to impaired drug delivery caused by the (i) lack of local vasculature at the tumour site and (ii) dense fibrotic tissue restricting penetration and distribution of drug within the tumour [2]. These factors cannot be easily overcome by systemic therapies because of dose related toxicities that preclude the use of high drug concentrations to achieve therapeutic response. In situ drug delivery strategies could overcome those difficulties. We fabricated pNIPAM-Au structures able to release Doxorrubicin on-demand upon short near- infrared (NIR) light exposure at determined time points over 3 days. The release from pNIPAM-Au stimulated was compared against passive release, free-drug, pNIPAM and pNIPAM-Au alone. The release profile of the samples showed an increment of drug release upon NIR exposure (~30%) compared to the non-stimulated samples. In vitro testing was carried using pancreatic cell lines BxPC-3, MIA PACA-2 and PANC-1 in 2D cell cultures and 3D spheroids models. The results showed that local delivery initiated by NIR triggered cell apoptosis thus providing a promising approach for localised management of PDAC.

Keywords

Pancreatic; Near-infrared; gold nanorods

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Abstract **#324**

TOWARDS A BETTER TISSUE REGENERATION THROUGH BIOMATERIAL-AIDED IMMUNOMODULATION: HARNESSING THE INNATE AND ADAPTIVE IMMUNE RESPONSES

Donghui Zhu[P][1]

[1] *Stony Brook University*

Corresponding author's email: donghui.zhu@stonybrook.edu

Immune system plays critical role in the process of tissue regeneration after injury, therefore, it is particularly important to take advantage of immune response to achieve the desired tissue restoration. In recent decades, biomaterial-aided immunomodulation has shown promising potential towards improved regeneration through harnessing the immune response during tissue repair process. A variety of factors, including physical signals, chemical cues and biological modulators, play great influence on immune cell functions and consequently regulate tissue regeneration. With respect to topological structure, rounded shape, large particle size and porosity, as well as high roughness are reported to reduce inflammation and resulted in less fibrosis when comparing to sharp, small and smooth structures. Other physical properties, such as low mechanical strength, low charge density and high hydrophilicity, help to inflammation resolution. Chemical carboxyl groups result in much less inflammatory cytokines comparing to amine-modified surface. Metal element such as magnesium is found to promote macrophage toward M2 phenotype (anti-inflammatory) polarization with increased production of anti-inflammatory cytokines. Besides the aforementioned methods, delivering biofactors to the target site with the help of biomaterials is considered a more effective way for immunomodulation. One strategy is to suppress pro-inflammatory cytokines production. An alternative way is to directly deliver anti-inflammatory agents such as IL-10 cytokine, genes, exosomes and even stem cells to help resolve inflammation and promote tissue regeneration. Based on these knowledges and strategies, further application of immunomodulation aided with biomaterials to regenerate bone, cartilage, muscle, skin and nervous system showed exciting prospect for tissue regeneration.

Abstract **#325**

THE MULTISTEP EFFECT OF MICROTUBULE STABILIZATION TO THE DIFFERENTIATION OF CHONDROCYTE CELL LINE

Jiawei Li[P][1], Ziying Sun[1], Zhongyang Lv[1], Xingquan Xu[1], Guoyang Sun[1], Huiming Jiang[2], Rongliang Wang[1], Rui Wu[1], Hu Guo[1], Qing Jiang[1], Dongquan Shi[1]

[1] State Key Laboratory of Pharmaceutical Biotechnology, Department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, 321 Zhongshan Road, Nanjing 210008, Jiangsu, PR China.

[2] Drum Tower of Clinical Medicine, Nanjing Medical University, Nanjing 210008, Jiangsu, PR China.

Corresponding author's email: 410998317@qq.com

Articular cartilage, distributed at the joint surface mainly consists of hyaline cartilage playing a role in load-bearing and lubricating. The formation of hyaline cartilage with healthy biological function is the main challenge in cartilage repair. Several studies reported that the regulation of cytoskeleton on chondrocyte differentiation by different mechanisms. In this study, the stability of microtubule (a member of the cytoskeleton) was positively correlated with cartilage-related gene expression in human osteoarthritis (OA) cartilages and the process of cartilage degeneration in the mouse OA model. The up-regulation of Ace-tubulin by docetaxel, a marker of the stable microtubule, improved the expression of collagen II, SOX9, and the SMAD3 (down-stream of the TGF-β pathway) in chondrocytes (CHs) and synovial mesenchymal stem cells (SMSCs), and promoted the cartilage regeneration in vivo. Then, we found that microtubule stabilization exhibited different mechanisms in the chondrogenesis of CHs and SMSCs. In the CHs, microtubule stabilization first promoted the yes associated protein (YAP), an important mechanotransduction protein, translocated to cytoplasm, and became p-YAP, which was inactive form. The increases of p-YAP kept the number and length of primary cilia and promoted cartilage-related proteins. As for SMSCs, microtubule stabilization directly led to the inhibition of YAP expression and primary cilia formation to prompt the TGF-β pathway and chondrogenesis differentiation. Taking together, the multistep effect of microtubule stabilization through the regulation of YAP and primary cilia to the different stages of the chondrocyte cell line, including CHs and MSCs promoted the hyaline cartilage phenotypes gene expression and cartilage regeneration.

Abstract **#326**

HYALURONAN-ENHANCED MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR MATRIX FOR CHRONIC SKIN WOUND REPAIR

Marisa Assunção[P][1,2], Chi Him Kendrick Yiu[1,2], Anna Blocki[1,2,3]

[1] *Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR*

[2] *School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR*

[3] *Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR*

Corresponding author's email: marisa.assuncao@link.cuhk.edu.hk

Chronic skin wounds are characterized by a persistent and dominant pro-inflammatory stimulus that delays tissue repair1. Cell-derived extracellular matrices (CDMs) hold a native tissue-like constitution and can be engineered in vitro to exhibit specific bioactivity and biophysical properties2,3. By combining the pro-regenerative secretome of bone-marrow mesenchymal stem cells (MSCs) with anti-inflammatory high-molecular weight hyaluronic acid (HA), we anticipated the generation of CDMs with enhanced healing potency for the treatment of chronic wounds.

Addition of HA to MSCs increased the deposition of fibronectin, a phenomenon we demonstrated to stem from HA's participation in fibronectin fibrillogenesis. To enhance ECM deposition further we used ficoll, a macromolecular crowder used in tissue engineering applications that by definition occupies space and enhances the kinetics of biological reactions4. The resulting CDMs, isolated at day 6, were rich in fibronectin, collagens I/IV, and heparan sulfate proteoglycans. To confirm that the CDMs exhibited the desired bioactivity, pro-inflammatory macrophages were exposed to CDMs in vitro. As a result, macrophages secreted lower levels of pro-inflammatory factors (i.e. tumor necrosis factor-α), as compared to controls without CDMs and CDMs without HA/ficoll.

Hydrogels formed with anti-inflammatory CDM were implanted in a murine chronic skin wound model, where they accelerated wound closure, as compared to empty hydrogels and CDMs with unspecific bioactivity.

In conclusion, we have elucidated one of HA's mechanisms of action, important to formulate fibronectin-rich platforms for tissue engineering. Moreover, the combination of MSC's secretome, HA and ficoll produced an anti-inflammatory CDM with enhanced properties capable of promoting wound healing.

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Abstract **#327**

ACTIVATION OF TRPV1 ALLEVIATES OSTEOARTHRITIS BY INHIBITING TYPE I MACROPHAGE POLARIZATION VIA CA2+/CAMKII/NRF2 SIGNALING PATHWAY

Zhongyang Lv[P][1], Xingquan Xu[1], Ziying Sun[1], Jiawei Li[1], Hu Guo[1], Yannick Xiao Fan Yang[1], Rui Wu[1], Qing Jiang[1], Dongquan Shi[1]

[1] *State Key Laboratory of Pharmaceutical Biotechnology, Department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, 321 Zhongshan Road, Nanjing 210008, Jiangsu, PR China.*

Corresponding author's email: zhongyanglv@163.com

Osteoarthritis (OA) is now well accepted as a low-grade inflammatory disease. Type I macrophage (M1) plays an important role in promoting synovitis during OA progression. Transient receptor potential vanilloid 1 (TRPV1), a pronociceptive non-selective cation channel, has been found expressed by M1 in OA synovium. The purpose of this project is to investigate whether targeting TRPV1 can inhibit M1 polarization and further retard OA progression. After stimulated by the TRPV1 agonist, capsaicin (CPS), the expression levels of M1 markers (IL-1β, IL-6, TNF-α, iNOS, and Cox2) in LPS-induced M1 cells were significantly down-regulated. Activating TRPV1 in M1 could promote nuclear factor E2-related factor 2 (Nrf2) nuclear localization, which was abolished by Nrf2 inhibitor, ML385. Under the stimulation of CPS, ML385 promoted M1 markers expression. CPS could stimulate Ca2+ influx in M1 cells. Both of using EDTA or Ca2+-free medium inhibited the CPS-stimulated Nrf2 nuclear localization. TRPV1 activation-induced Ca2+ influx promoted the phosphorylation of Ca2+ /calmodulin-dependent protein kinase II (CaMKII) in a time-dependent manner, and further promoted Nrf2 nuclear localization, which was abolished by CaMKII antagonist, KN-93. In vivo, compared with the OA group, intra-articular injection of CPS could reduce the joint swelling and osteophyte formation significantly. MicroCT and histological staining of 4- and 8-week rat knees showed less osteophyte formation, cartilage loss, and inflammatory cells infiltration in the OA+CPS group. In conclusion, we found that activating TRPV1 can inhibit M1 polarization via the Ca2+/CaMKII/Nrf2 signaling pathway and then alleviate OA in vivo, these findings provide a potential therapeutic target for OA.

Abstract **#328**

WORKING TOWARDS A REGENERATIVE THERAPY FOR OSTEOARTHRITIS: INFLUENCE OF STEM CELLS

Jiao Jiao Li[P][1,2], Christopher Little[2]

[1] *School of Biomedical Engineering, University of Technology Sydney*

[2] *Kolling Institute, University of Sydney*

Corresponding author's email: jiaojiao.li@uts.edu.au

Background: Osteoarthritis is incurable and a leading cause of disability worldwide. Mesenchymal stem cells (MSCs) have recently been used to treat osteoarthritis due to their anti-inflammatory and pro-regenerative characteristics. Interestingly, the few clinical trials utilising MSCs to treat knee osteoarthritis have not demonstrated consistent benefits[1]. This study aims to understand the variable efficacy of stem cell therapy using an in vitro model of a human osteoarthritic joint.
Methods: MSCs were co-cultured with human synovial fibroblasts (HSFs) isolated from osteoarthritic tissues, for 21 days in growth, osteogenic and chondrogenic media (simulating relevant conditions in the joint). Cell interactions were assessed using RT-PCR (n=4) and histology (n=2).
Results: MSCs co-cultured with osteoarthritic HSFs showed increased inflammation (MMP2, ADAMTS5) and impaired ability to form new bone (reduced BSP, SPP1 expression and calcium deposition) and cartilage (reduced COL2A1, ACAN expression and proteoglycan deposition) at 21 days, suggesting that the osteoarthritic joint is an inhibitory environment that reduces the therapeutic effects of MSCs. Furthermore, short-term (3 days) exposure of the osteoarthritic HSFs to MSCs ('pre-conditioning') was insufficient for sustained modifications to their diseased phenotype. The osteoarthritic HSFs, whether previously exposed to MSCs or not, had similar expression profiles of inflammatory markers, and also similar negative effects on MSCs, including inflammatory marker upregulation (e.g. IL-8, ADAMTS4) and impaired chondrogenesis.
Conclusion: Diseased cells in an osteoarthritic joint can create an inhibitory environment that negatively affects the therapeutic effects of MSCs following injection. Future regenerative therapies for osteoarthritis may benefit from using the secreted products of stem cells.

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Abstract **#329**

DEVELOPMENT OF 3D- SCAFFOLD USING BIOINK DERIVED FROM DECELLULARIZED BONE WITH CALCIUM PHOSPHATE CEMENT FOR BONE TISSUE REGENERATION

Niloofar Kalantari[1,2], Mohammadreza Baghaban Eslaminejad[1], Samaneh Hosseini[1,3]

[1] *Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran*

[2] *University of Science and Culture, Tehran, Iran*

[3] *Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and |Technology, ACECR, Tehran, Iran*

Corresponding author's email: niloofarkalan@gmail.com

Critical-sized bone defects require additional interventions to restore. 3D-bioprinting has emerged as a promising approach in bone tissue engineering to recapitulate a living tissue with greater precision. However, development an ideal bioink with high osteogenic potential remains challenging. Herein, we developed an ECM-based hydrogel employing decellularized bovine bone and calcium phosphate cement(CPC) as a bioink for 3D-bioprinting. A demineralization/decellularizion protocol was set up and the decellularized bone ECM (dECM) was further characterized in terms of morphology, collagen and DNA contents. dECMhydrogel combined with different concentrations of CPC was mechanically and rheologically analyzed to achieve the appropriate bioink based on physical properties. Adipose derived mesenchymal stem cell (ADMSCs)-laden hydrogels were then assessed using Live/Dead, real-time PCR and histological analysis to evaluate cell proliferation and osteogenic capacity of fabricated hydrogel. H&E staining and DNA contents demonstrated that our protocol could efficiently decellularize bone tissue and the number of cells had extremely decreased. Collagen assay showed that the decellularization process did not affect the amount of collagen present in the tissue. As expected, CPC increased the mechanical strength of hydrogel. The rheological analysis confirmed gel behavior trait as well as shear-thinning property of bioink, which is necessary for 3D-printing. Cellular experiments revealed the viability of cells and their differentiation into osteogenic lineage in hydrogel after 21 days. In sum, these findings highlight the promising potential of this hydrogel as a suitable bioink for 3D-bioprinting and hold great promise as a novel therapeutic approach in bone tissue engineering for future studies.

Keywords

Bone tissue engineering ; Bioink; Decellularization

Abstract **#330**

IMPROVED LONG-TERM VOLUME RETENTION IN FAT GRAFTING USING 3D PRINTED PCL SCAFFOLDS WITH BIONIC STRUCTURE

Wanting Bao[P][1,2,3]

[1] *Shanghai Key Laboratory of Tissue Engineering*

[2] *Shanghai Ninth People's Hospital*

[3] *Shanghai Jiao Tong University School of Medicine*

Corresponding author's email: bigbaobao@sjtu.edu.cn

Background: Autologous fat grafting has played an increasingly important role in breast reconstruction. However, mature adipocytes are sensitive to stress and hypoxia, which causes a high rate of necrosis, absorption, and fibrosis. The anatomical structure of breasts shows that adipose tissue is surrounded by suspensory ligaments. These ligaments provide mechanical support to keep the special shape of breasts. Inspired by this, bionic scaffolds are assumed to be beneficial for fat grafting.

Objective: To evaluate the effect of bionic scaffolds on long-term volume retention in fat grafting and to explore potential mechanisms.

Method: PCL scaffolds were fabricated by 3D-printing technique. Liposuction fat was compounded with scaffolds and implanted subcutaneously in nude mice. After 2,4,8, and 12 weeks, gross and histological observations were performed to evaluate the survival of adipose tissue. The percentages of adipocytes, vacuoles, and fibrosis were estimated through HE and Masson staining. The adipocyte viability was analyzed by Perilipin-1 and vascularization by CD31. IHC and qPCR were used to detect the expression of HIF-1α and VEGF.

Results: The retention rate of the experimental group (fat with scaffolds) was significantly higher than that of the control group (fat without scaffolds). The experimental group exhibited fewer vacuoles, less fibrosis, and elevated vascularization. The lower expression of HIF-1α in the experimental group indicated that hypoxia was reduced at early stage.

Conclusion: The scaffold can provide mechanical support to resist skin tension and tissue internal pressure. Therefore, the state of hypoxia can be alleviated, which improves the long-term retention rate of implanted fat.

Keywords

Fat grafting; Scaffold; Hypoxia

Abstract **#331**

IN VITRO MODELS OF FIBROTIC CARDIAC TISSUE BASED ON ELECTROSPUN BIOARTIFICIAL SCAFFOLDS

Gerardina Ruocco[P][1,2,3], Irene Carmagnola[1,2,3], Alice Zoso[1,2,3], Mattia Spedicati[1,2,3], Valeria Chiono[1,2,3]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *POLITO Biomedlab, Politecnico di Torino, Turin, Italy*

[3] *Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Italy*

Corresponding author's email: ruoccogerardina1@gmail.com

Myocardial Infarction causes the irreversible loss of billions of cardiomyocytes and the beginning of reparative healing, resulting in excessive deposition of highly crosslinked collagen by Myofibroblasts (MyoFs), fibrosis, stiffening, and cardiac function impairment [1]. Currently, there is no effective treatment to reverse cardiac fibrosis, so heart transplantation remains the only available clinical approach. In this context, in vitro models able to reproduce post-infarct microenvironment for preclinical drug screening are needed.

In this work, an engineered in vitro 2D-model of human cardiac fibrotic tissue was designed in order to reproduce small extensions of early stage myocardial fibrosis. In detail, bioartificial scaffolds based on polycaprolactone (PCL) and Gelatin (G) were fabricated. PCL scaffolds were prepared by electrospinning having random morphology, narrow nanofibers size distribution and interconnected porosities, closely mimicking the architecture of native extracellular matrix (ECM). To further mimic the ECM chemical composition, PCL scaffolds were surface modified using a mussel-inspired strategy: G (Type A) was grafted exploiting an adhesive 3,4-Dihydroxy-D,L-phenylalanina (DOPA) precoating. Physicochemical and mechanical characterizations were performed to assess functionalization success and coating stability. Human Cardiac Fibroblasts (HCFs) isolated from human ventricle were then cultured on the scaffolds at a density of 7x104 cells/cm2. Their adhesion, functional organization and biomatrix deposition were analysed at long-term culture times. Biological validation demonstrated that bioartificial scaffolds promote HCFs adhesion, spreading and deposition of the typical cardiac ECM proteins.

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Abstract **#332**

SURFACE GELATIN-COATING ON 3D PRINTED PLA SCAFFOLD FOR LIVER TISSUE ENGINEERING

Elnaz sadat Mirdamadi$_P$[1], Zahra Khosrowpour[2], Mazaher Gholipourmalekabadi[2,3], Mehran Solati-Hashjin[1]

[1] *BioFabrication Lab (BFL), Department of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran 1591634311, Iran*

[2] *Cellular and Molecular Research Centre, Iran University of Medical Sciences, Tehran 1449614535, Iran*

[3] *Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran 1449614535, Iran*

Corresponding author's email: elnazmirdamadi258@gmail.com

The liver as a vital organ can suffer from various diseases that make the survival of the patient critical. The only current treatment is liver transplantation, which has many challenges. One alternative approach is liver tissue engineering to using scaffolds assisting or replacing the damaged liver. For the liver, as soft tissue, biopolymers coupled with natural hydrogels are commonly used. In this study, PLA (Poly Lactic Acid) was used as the input of a Fused Deposition Modeling-FDM 3D printing machine to investigate the effect of gelatin surface coating methods on HepG2 cells. The 3D printed scaffolds were coated with 1 and 2% gelatin by three methods “Duplex, Entrapment, and M-Entrapment.” The printing process and coated gelatin were structurally examined by micro and macro images to compare the homogeneity of the coating. In vitro toxicity MTT assay was performed by HepG2 cell culture on scaffolds that showed no toxicity. Also, cell morphology was observed by SEM images, in gelatin-free samples, the cells were aggregated due to low affinity for adhesion to a PLA surface. By comparing cell adhesion and surface homogeneity, the M-Entrapment method was considered to be a more hostile surface for cells.

Keywords

PLA; Gelatin; Scaffold

Abstract **#333**

MULTIFUNCTIONAL INJECTABLE HYDROGELS TRIGGERING DIRECT REPROGRAMMING OF ADULT HUMAN CARDIAC FIBROBLASTS INTO INDUCED CARDIOMYOCYTES

Valeria Chiono[P][1,2,3], Camilla Paoletti[1,2,3], Letizia Nicoletti[1,2,3], Giulia Tarricone[1,2,3], Chiara Tonda-Turo[1,2,3], Clara Mattu[1,2,3], Ugo Ala[4], Carla Divieto[5], Daria Nurzynska[6], Franca Di Meglio[6]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *POLITO Biomedlab, Politecnico di Torino, Turin, Italy*

[3] *Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Italy*

[4] *Department of Veterinary Science, University of Turin, Turin Italy*

[5] *Istituto Nazionale di Ricerca Metrologica, Advanced Materials Metrology and Life Science, Turin, Italy*

[6] *Department of Public Health, University of Naples Federico II, Naples, Italy*

Corresponding author's email: valeria.chiono@polito.it

Direct reprogramming of cardiac fibroblasts into induced cardiomyocytes (iCMs) represents a promising strategy to restore cardiac functions after myocardial infarction [1]. Previous studies have identified a combination of microRNA-mimics (miRcombo: miR-1, 133, 208, 499) able to directly reprogram mouse fibroblasts into iCMs [2]. Respect to methods based on the expression of cardiac transcription factors via viral vectors, microRNAs can be delivered into the cell cytoplasm by safer nanocarriers, paving the way towards in situ treatments [3]. The work was aimed at the development of injectable hydrogels for in situ delivery of microRNA/nanocarriers, further provided with biochemical cues enhancing direct reprogramming of adult human cardiac fibroblasts (AHCFs) into iCMs. Initially, miRcombo was found to trigger transdifferentiation of AHCFs into iCMs, inducing cardiac markers upregulation (11% cTnT-positive cells after 15 days) and cardiac-like functionality (38% cells with calcium transients after 30 days) [4]. Cardiac markers were further upregulated for miRcombo-transfected cells cultured on 2D plates coated with gelatin, laminin or AHCF-derived biomatrix [5] with biomatrix triggering significantly higher cTnT expression. As Algisyl (based on alginate) and Ventrigel (based on porcine extracellular matrix) [6] are the only hydrogels under clinical trials for post-infarct treatment, we prepared injectable hydrogels based on alginate functionalized with RGD and/or biomatrix with dual (ionic and click-chemistry-driven) crosslinking ability. Hydrogels loading lipoplexes/miRcombo [7] are under study for their ability to directly reprogram AHCFs in vitro.

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Abstract **#335**

POLY(2-HYDROXYETHYL METHACRYLATE) (PHEMA)-BASED HYDROGELS AS A SYNTHETIC PLATFORM TO STUDY PERIPHERAL NERVE REGENERATION

Sergio Oliveira Formoso[P][1], Adam J. Reid[2], Lee A. Fielding[1]

[1] *Department of Materials, School of Natural Sciences, The University of Manchester, Manchester (UK), M13 9PL*

[2] *Blond McIndoe Laboratories, Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health. University of Manchester, Manchester (UK), M13 9PL*

Corresponding author's email: sergio.oliveiraformoso@manchester.ac.uk

PHEMA-based hydrogels are well-studied materials for tissue engineering due to their biocompatibility, controllable synthesis and tuneable physicochemical properties [1]. These properties allow PHEMA hydrogels to be suitable candidates for use in peripheral nerve regeneration. This work shows the synthesis, characterisation and initial biocompatibility studies of a range of PHEMA-based hydrogels. Homogeneous hydrogels with storage moduli in the range of 8-12 MPa were obtained via redox-initiated free-radical polymerisation, with maximum liquid uptakes of approximately 200 % obtained over 48h without disturbing the bulk dimensions. Copolymerisation with gelatin methacryloyl (GelMA) in concentrations up to 20 % w/w allowed the improvement of both degradability and biocompatibility [2]. GelMA containing hydrogels displayed sustained losses in mechanical properties after incubation in collagenase I solution, showing a 5 MPa decrease in storage modulus and increased liquid uptake. Functionalisation of gel surfaces with proteins present in the peripheral nerve extracellular matrix allowed improvement in cell viability and proliferation for adipose-derived stem cells, Schwann cells and DRG populations [3]. Therefore, the aforementioned PHEMA-based hydrogels are envisioned to be used as synthetic platforms to investigate these important cell populations in peripheral nerve regeneration and ultimately their suitability as artificial nerve conduits.

Keywords

synthetic polymers; polymer characterization; biocompatibility

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Abstract **#336**

FABRICATION AND EVALUATION OF A MACROPHAGE-CONTAINING SKIN SUBSTITUTE: AN ANIMAL STUDY

Sadjad Khosravimelal$_P$[1,2], Samane Eftekhari[1,2], Mazaher Gholipourmalekabadi[1,3], Ali Samadikuchaksaraei[1,2,3]

[1] *Cellular and Molecular Research Centre, Iran University of Medical Sciences, Tehran, Iran.*

[2] *Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran.*

[3] *Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*

Corresponding author's email: sadjadkhosravi@yahoo.com

Studies on new modalities that can accelerate the healing process of skin injuries are still in progress. One of the attractive areas in this field, which has shown promising results, is application of cellular and tissue engineered products. The aim of this study is to use a macrophage-containing tissue engineered amniotic membrane as a dermal substitute in an animal model. After written consent, human amniotic membrane (HAM) was collected at the caesarian section in the operating room. HAM was decellularized using a detergent-based method. Macrophages were extracted from peritoneum of C57 male mice and characterized using CD68, CD163 and CD206 markers by flow cytometry and then, were seeded on the decellularized HAM. The cytobiocoampatibilty of the scaffolds were investigated using MTT assay. Afterwards, the scaffolds were implanted on the back skin of C57 male mice. Results were analyzed on days 1, 3, 7 and 28 post-injuries for the quality of healing using both visual scale and H&E staining of histological sections. The results have shown significant difference in acceleration and promotion of wound healing in HAM scaffolds seeded with macrophages compared to other experimental groups (HAM alone and control) on days 3, 7 and 28 post-injuries. Accordingly, the macrophage-containing skin substitute could be considered as a potential modality for acceleration of skin wound healing.

Keywords

Skin substitute; amniotic membrane; macrophage

Abstract **#337**

HOW PRESERVATION PERIOD AFFECT THE BIOLOGICAL PROPERTIES OF ACELLULAR PLACENTAL SPONGE PATCHES?

Sadjad KhosravimelalP[1,2], Fatemeh Asgari[1,3], Zahra Aliakbar Ahovan[4], Morteza Koruji[1,3], Ali Shirani[1,5], Ali Hashemi[4], Hatef Ghasemi Hamidabadi[6,7], Narendra Pal Singh Chauhan[8], Lorenzo Moroni[9], Rui L Reis[10], Subhas C Kundu[10], Mazaher Gholipourmalekabadi[1,5,11]

[1] Cellular and Molecular Research Centre, Iran University of Medical Sciences, Tehran, Iran.
[2] Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran.
[3] Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
[4] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[5] Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.
[6] Immunogenetic Research Center, Department of Anatomy & Cell Biology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
[7] Department of Anatomy & Cell Biology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
[8] Department of Chemistry, Faculty of Science, Bhupal Nobles' University, Udaipur, Rajasthan, India
[9] Complex Tissue Regeneration Department, Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands
[10] 3Bs Research Group, I3Bs - Research Institute on Biomaterials, Biodegradable and Biomimetics. Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, AvePark, Guimaraes, Portugal.
[11] Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medicine Sciences, Tehran, Iran.
Corresponding author's email: sadjadkhosravi@yahoo.com

Understanding the effects of storage time and preservation method on the biological and physical characteristics of biomaterials have always been a concern in tissue engineering and regenerative medicine. In the present study, we evaluated the impacts of storage time on decellularized placental sponges (DPS) in vitro and in vivo. For this purpose, the scaffolds were incubated for one, three and six months after decellularization at -20 °C and their antibacterial, angiogenic and biocompatibility properties were examined on each time intervals. The decellularized samples demonstrated a uniformed structure with interconnected networks in comparison with intact placental scaffolds. The preservation time did not affect the cyto-biocompatibility of DPSs against Hu02 foreskin fibroblast cells and also collagen and VEGF contents. Compared to other experimental groups, the six months preserved samples showed a decreased neovascularization assessed by chorioallantoic membrane assay and subcutaneous implantation on NMRI male mice. Also, in six months DPSs, the levels of CD4+ and CD68+ infiltered cells were elevated dramatically compared to other DPSs. The antibacterial characteristics of the samples decreased over the storage time. According to the results, the three months-stored DPSs were considered as the optimum preserved samples due to maximum antibacterial activity and increased new blood vessel formation. This presented storage method can be considered for other decellularized placental products used in biomedical applications.

Keywords
placenta; decellularization; preservation

Abstract **#339**

IN SITU TISSUE REGENERATION FROM CONCEPT TO CLINICAL TRIALS

May Griffith$_P$[1,2]

[1] *University of Montreal, Montreal, QC, Canada*

2 Maisonneuve-Rosemont Hospital Research Centre

Corresponding author's email: May.Griffith@umontreal.ca

The most common treatment for corneal blindness is donor cornea transplantation, but a severe worldwide shortage of donor corneas leaves an estimated 12.7 million patients awaiting transplantation.[1] Donor transplantation needs to be supplemented with bioengineered solutions in order to reach the millions of patients. In Fagerholm et al. [2,3], we showed that cell-free, bio-responsive hydrogels grafted as corneal implants induced the patients' own endogenous stem cells to regenerate their corneas. We have subsequently shown that inflammation suppressing polymers can be integrated into the implants for use in patients with more severe pathologies [4]. Most recently, to address corneal perforation and conditions that can be treated by an anterior lamellar graft, we developed a synthetic, biocompatible and adhesive liquid hydrogel (LiQD Cornea) that would be used in place of toxic cyanoacrylate glue used to seal perforations, as well as human donor tissue for anterior lamellar graft replacements.[5] LiQD cornea is applied as a liquid, but quickly adheres and gels within corneal tissue defects – a bit like a dental filling. Then its similarity at a molecular level to a natural tissue framework promotes tissue regeneration, treating corneal perforations effectively without the need for transplantation. Further, due to the synthetic nature of the material, risks associated with immune rejection and disease transmission are also reduced compared to natural products in both our solid and liquid implants. We see LiQD Cornea potentially allowing for clinical application in outpatient clinics instead of operating theaters, maximizing practicality, and minimizing healthcare costs.

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Abstract **#340**

IN SILICO AND IN VITRO ANALYSIS OF MECHANOBIOLOGICAL EFFECTS ON LIVING CELLS DURING NOZZLE-BASED BIOPRINTING PROCESS

Ramin Nasehi[P][1], Carsten Ludwig[1], Sanja Aveic[1], Horst Fischer[1]

[1] *Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany*

Corresponding author's email: rnasehi@ukaachen.de

Bioprinting is a technology with high potential in enabling the tailored arrangement of different cell types in a tissue mimetic spatial manner. However, it is not yet completely understood how the printing process affects the mechanobiological cues of the cells. To characterize better how the bioprinting process impacts the bioink embedded cells, we simulated the fluid flow inside the nozzle. Two well-established bioprinting methods (drop-on-demand and microextrusion) were analyzed using FEM and data were validated experimentally. Our simulation results reveal that in drop-on-demand bioprinters, the flow is mostly in the transient regime and the onset and duration of the deformation of the cells in the nozzle is in order of microseconds (~500-10000µs). Using the microextrusion technique, the flow regime is in steady-state condition and onset of deformation is in the same order; however, its duration is comparatively higher (~seconds) and still a function of needle length and flow rate. While cells are continuously under the effect of hydrostatic pressure during entire duration of printing, the shear stress imposed on the cells is verified just at the moment that bioink is ejected from the nozzle (micro/milliseconds). Preliminary experiments show that the application of long-term hydrostatic pressure affects the expression level of genes involved in focal adhesions and ATP-level. In conclusion, our results confirm that the bioprinting process affects specific functional cell characteristics. These findings could guide the development of printer nozzles with cell-preserving features and may be adopted as initial strategies for optimizing the technology used for cell-laden hydrogels printing.

Abstract **#341**

A CLINICALLY APPLICABLE COCHLEAR ELECTRO-ANATOMICAL MODEL USING 3D PRINTING AND MACHINE LEARNING

Iek Man Lei[P][1], Chen Jiang[2], Chon Lok Lei[3], Simone de Rijk[2], Yu Chuen Tam[4], Manohar Bance[2], Yan Yan Shery Huang[1]

[1] *Department of Engineering, University of Cambridge, UK*

[2] *Department of Clinical Neurosciences, University of Cambridge, UK*

[3] *Department of Computer Science, University of Oxford, UK*

[4] *Emmeline Centre for Hearing Implants, Addenbrookes Hospital, Cambridge, UK*

Corresponding author's email: iml28@cam.ac.uk

Cochlear implants (CI) have brought sound to millions with profound hearing loss. However, today's CI performance is still limited by the imprecise control of electrical stimulus and, thus, 'blurring of the perceived sound'. The electrical spread characteristics in cochleae depends on the CI positions in cochleae, and the electrical and anatomical features of human cochleae, which cannot be fully replicated in animal models or human cadaver models. Here, we report the development of a library of 3D printed electro-anatomical cochlear phantoms via constructing human representative cochlear geometry in a tuneable electro-mimicking bone matrix. By measuring the stimulation spread characteristics of all 3D printed phantoms using electric field imaging and deploying a machine learning model to simulate the experimental data, our platform can comprehensively emulate the stimulation spread characteristics resulting from the wide-ranging electro-anatomical features of the human cochleae. Validated with clinical data, our platform can correctly predict patient intra-cochlear stimulation spread characteristics with over 85% accuracy. We then exploited this platform to decipher the intricate relationship between the stimulation spread characteristics and the cochlear electro-anatomical features, hence predicting the patient cochlear features that will be more prone to the stimulation spread. Further to this, our platform can reproduce patient's stimulation spread characteristics via a physical 3D printed phantom. We anticipate that such platform will be particularly beneficial in facilitating the development of CIs. Our methodology can be readily transferrable to other anatomical sites, paving the way for advanced testing platform to screen new electronic implants.

Abstract **#343**

3D PRINTED JANUS SCAFFOLDS FOR NANOVIBRATION-DRIVEN BONE REGENERATION

Sandra Camarero-Espinosa$_P$[1,2], Lorenzo Moroni[2]

[1] *POLYMAT*

[2] *Maastricht University*

Corresponding author's email: sandra.camarero@polymat.eu

Physical stimulation of cell cultures activates mechanotransduction pathways that result on the modulation of cellular functions such as migration, differentiation or survival. Despite the great potential shown by in-vitro models, these are generally based on sophisticated systems with compromised potential to be exploited in-vivo, being the main short-coming the incapability to apply the stimuli “externally” and on a controlled manner. Here, we present the first report on dynamic 3D printed scaffolds that can be remotely actuated via application of ultrasounds, resulting on a controlled mechanical nanovibration that is transmitted to neighboring cells. Dynamic scaffolds are based on Janus structures that are spontaneously formed via phase-segregation of polycaprolactone (PCL) and polylactide (PLA) blends during the 3D printing process. Janus scaffolds behave as ultrasound transducers (acoustic to mechanical) with a mechanically active, PLA and a backing material (PCL). Remote stimulation of Janus scaffolds led to an enhanced cell proliferation, matrix deposition and osteogenic differentiation of cultured bone marrow derived stromal cells via formation of voltage-gated calcium ion channels.

Abstract **#344**

CARTILAGE-ON-CHIP: A RELIABLE MODEL TO MIMIC CHONDROCYTE PERICELLULAR STRUCTURE

Carlo Alberto Paggi[1], Jan Hendriks[1], Liliana Moreira Teixeira[1,2], Séverine Le Gac[1], Marcel Karperien[1]

[1] *University of Twente*

[2] *Utrecht University*

Corresponding author's email: c.a.paggi@utwente.nl

Chondrocytes residing in the articular cartilage are surrounded by a pericellular matrix which sustain the compression and shear strain forces exerted during movement. Little is known about the development of this native pericellular matrix. Here, we employed a cartilage-on-chip platform which allow application of multi-modal mechanical stimulation [1] to identify the type of mechanical stimulation required for the formation of this protein shell around chondrocytes grown in an agarose matrix (2% w/v). After 7 days of culture in static conditions, human chondrocytes were cultured for other 7 days in static or dynamic conditions (compression or compression & shear stress) (1 Hz for 1 hour a day). Medium was collected from the chip to quantify IL-6 using enhanced SPRi [2]. IL-6 secretion by the mechanically stimulated samples was significantly higher compared to the static conditions for the first 7 days, suggesting a role of mechanical stimulation in IL-6 production. In both dynamic and static conditions IL-6 secretion reduced over time suggesting cell adaptation to their on-chip environment. Aggrecan, Col II & Col VI immunofluorescence revealed higher pericellular matrix formation in dynamic conditions compared to static culture. Interestingly, cells exposed to the combined compression and shear strain, compared to only compression, presented double cells and higher Col II matrix deposition, both characteristics of healthy articular cartilage. Alcian blue stain revealed pericellular matrix formation (2,5 µm thickness) in both dynamic conditions further supporting the development of a native pericellular matrix. Our cartilage-on-a-chip platform supports the formation of quasi-native pericellular matrix.

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Abstract **#345**

3D PRINTING OF POLYHYDROXYALKANOATES FOR CELL CULTURE AND TISSUE REPAIR APPLICATIONS

David Alexander Gregory$_P$[1], Annabelle Fricker[1], Emmanuel Asare[1], Ipsita Roy[1,2]

[1] *Department of Material Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK*

[2] *National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, United Kingdom*

Corresponding author's email: d.a.gregory@sheffield.ac.uk

In recent decades tissue engineering has seen a revolution in the development of novel materials and bio-fabrication methods. The move from classical 2D to 3D scaffolds, which mimic the natural habitat of cells, is important and novel 3D printing technologies are constantly being developed.[1] 3D printing (additive manufacturing) is becoming one of the preferred methods to generate complex scaffolds for a large variety of biomedical engineering applications e.g. wound healing patches, patient specific implants and devices. Successful implementation of these scaffolds depends on the biomaterials, their properties and the manufacturing technologies used. In this context, Polyhydroxyalkanoates (PHAs) have exhibited exceptional value.[2-3]

PHAs are natural biopolymers that are sustainably produced via bacterial fermentation and have been steadily gaining popularity for biomedical engineering applications due to their excellent biocompatibility towards a large variety of cell types.[4] Moreover, their tuneable mechanical properties, as well as non-toxic biodegradability via surface erosion make them excellent candidates for scaffold materials.[2] Furthermore, PHAs are easily processable and therefore excellent materials for additive manufacturing.

Here, we demonstrate the successful 3D printing of PHAs via fused deposition modelling of various CAD-designed structures for use in tissue engineering.[1] Further, we demonstrate the ability to tune the mechanical properties of our scaffolds via both material properties as well as by altering the design of the printed structures. Short-term degradation studies are performed with the printed constructs and cell compatibility is assessed. Finally, the prospects of PHA-printed scaffolds for next generation in-vitro and in-vivo studies are discussed.

Keywords

Polyhydroxyalkanoates; 3D Printing; 3D scaffolds

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Abstract **#346**

SYNOVIAL MEMBRANE ON CHIP: A NEW TOOL TO STUDY REUMATHOID DISEASES

Carlo Alberto Paggi[1], Mariia Zakharova[1], Loes Segerink[1], Liliana Moreira-Teixeira[1,2], Séverine Le Gac[1], Marcel Karperien[1]

[1] *University of Twente*

[2] *Utrecht University*

Corresponding author's email: c.a.paggi@utwente.nl

The synovial membrane preserves the integrity of the knee joint and is a key player in the development of rheumatoid diseases. These tissue is mainly composed of synovial fibroblasts and macrophages. During joint movement cells in the synovial membrane are exposed to mechanical stress. Despite this well known fact, little is known about the synovial tissue response to mechanical stimulation. Here, we present a synovial-membrane on-chip platform that combines both synovial fibroblasts and macrophages and allows multi-directional mechanical stimulation (inspired by [1]), by independently addressing 6 actuation chambers deforming a flexible membrane covered by a monolayer of fibroblasts and macrophages. The system exerts up to 60% of deformation which allows both physiological and hyper-physiological stretching of the cell-covered membrane. Negative pressure (-350 mbar) can be applied to each of the 6 actuation chambers independently or in combination, allowing multi-stretching patterns. After a collagen coating of the flexible porous PDMS membrane, synovial fibroblasts were seeded and cultured for 7 days in static conditions (confluency reached after 1 day). Application of hyper-physiological stretching of the membrane, but not physiological stretching for 1 hour a day (1 Hz) induced a high level of cell death of synovial fibroblasts cell four days later. In addition the system was compatible with co-culture of synoviocytes and macrophages (cell viability up to 14 days). With this innovative organ-on-chip platform we are, for the first time, able to recapitulate and study the mechanical actuation of an engineered synovial membrane in healthy and diseased conditions.

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Abstract **#347**

FABRICATION OF CELL-INSTRUCTIVE INTERFACES USING DNA MODIFIED MSNS FILMS

Xingzhen Zhang$_P$[1], Pamela Habibovic[1], Sabine van Rijt[1]

[1] *Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University*

Corresponding author's email: xingzhen.zhang@maastrichtuniversity.nl

Stem cells, with their inherent ability to self-renew and differentiate, play an important role in tissue development, tissue homeostasis, and wound repair. Due to these properties, stem cells offer great potential in the field of tissue regeneration. However, their regenerative ability and cellular process is controlled by multi-factorial signals presented in their natural resident microenvironments. Therefore, to effectively direct tissue regeneration in vitro, the development and engineering of smart interfaces that can finely regulate stem cell behavior is highly desired. Mesoporous silica nanoparticles (MSNs) represent an interesting material to create bio-interfaces for enhanced regenerative application by enabling close control over surface physiochemical properties. Moreover, the mesoporous structure and versatile surface functionalization properties make MSNs excellent materials for cargo-loading and controlled drug delivery.

In this project, we aim to use DNA functionalized MSNs (MSNs-ssDNA) to create 2D bioengineered films to guide stem cell function, where short DNA duplex can serve as a highly programmable linker to capture specific bioactive ligands over the MSNs surface and MSNs can be used as biomolecules delivery carrier. For this, monodisperse MSNs with a narrow size distribution around 200 nm have been synthesized. Oligonucleotides were grafted to MSNs and the DNA graft ratios were quantified by Nanodrop. MSNs-ssDNA based film was further prepared and characterized by Scanning Electron Microscope. This platform is useful for diverse applications in tissue engineering. It can be applied as highly tailored biomimetic interface to elicit desired cell response, or as coating materials to improve biological properties and tissue integration of implants.

Abstract **#348**

ORGANOPHOSPHATE DETOXIFICATION BY MEMBRANE-ENGINEERED RED BLOOD CELLS

Paige Smith$_P$[1], Leran Mao[2], Kaustubh Sinha[1], Alan Russell[1,2]

[1] *Department of Biological Sciences, Carnegie Mellon University*

[2] *Department of Chemical Engineering, Carnegie Mellon University*

Corresponding author's email: pnsmith@andrew.cmu.edu

Biotherapeutics have achieved global economic success due to their high specificity towards their drug targets, providing exceptional safety and efficiency. The ongoing shift away from small molecule drugs towards biotherapeutics heightens the need to further improve the pharmacokinetics of these biological drugs. Three pervasive obstacles that limit the therapeutic capacity of biotherapeutics are proteolytic degradation, circulating half-life, and the development of anti-drug antibodies. These challenges can culminate in limited efficiency and consequently warrant the need for higher drug doses and more frequent administration. We have explored the coupling of biotherapeutics to long-lived and biocompatible red blood cells (RBCs) to address limited pharmacokinetics. Butyrylcholinesterase (BChE), for example, provides prophylactic protection against organophosphate nerve agents (OPNAs), yet the short circulation life of the drug requires extraordinary doses. Herein, we report the rapid and tunable chemical engineering of BChE to RBC membranes to create a cell-based delivery system that retains the enzyme activity and enhances stability. In a three-step process that first pre-modifies BChE with a cell-reactive polymer chain, primes the cells for engineering, and then grafts the conjugates to the cells, we attached over 2 million BChE molecules to the surface of each RBC without diminishing the bioscavenging capacity of the enzyme. Critically, this membrane-engineering approach was cell-tolerated with minimal hemolysis observed. These results provide strong evidence for the ability of engineered RBCs to serve as an enhanced biotherapeutic delivery vehicle.

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Abstract **#350**

THE EFFECT OF DIFFERENT BIOPHYSICAL FORCES ON THE OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS (ASCS)

Lara Lingens[P][1], Tim Ruhl[1], Gerrit Freund[1], Justus P. Beier[1], Rebekka Götzl[1]

[1] *Department of Plastic Surgery, Hand and Burn Surgery, University Hospital RWTH Aachen*

Corresponding author's email: llingens@ukaachen.de

Human adipose stem cells (ASCs) hold huge potential for regenerative medicine approaches, including osteogenic regeneration. In vivo, osteogenic differentiation is dependent as well as stimulated by the impact of different bio-physical stimuli, such as mechanical manipulation and oxidative stress . In our study, we tested the effects of different bio-physical impacts to enhance the osteogenic differentiation in ASCs.

During 14 days of osteogenic differentiation, ASCs were intermittently stimulated with hypergravity (10x g, 20x g, 30x g, 40x g) to create a mechanical force acting on the cells. In a second experiment ASCs underwent osteogenic differentiation at hypoxic conditions (3% O2, 8% O2, 20% O2). In both approaches, the calcification using cresolphtalein staining, and the enzymatic activity of alkaline phosphatase (ALP) was analysed to detect and quantify the differentiation process.

Hypergravity increased significantly the ALP activity as well as the cellular calcification with highest values at 20x g and 30x g. Furthermore, we found a significant increase of calcification and ALP activity at hypoxia of 3% and 8% with highest values at 8%. Also, the proliferation was significantly higher under hypoxic conditions compared to standard culture.

The stimulation of ASCs during osteogenic differentiation with hypergravity and hypoxia show new pathways to possibly improve osteogenic tissue engineering or osteogenic regeneration using ASCs.

Abstract **#352**

UNDERSTANDING THE DYNAMICS OF PRIMITIVE ENDODERM PATTERNING USING STEM CELL-BASED EMBRYO MODELS

Vinidhra Shankar$_P$[1], Clemens van Blitterswijk[1], Erik Vrij[1], Stefan Giselbrecht[1]

[1] *Maastricht University*

Corresponding author's email: v.shankar@maastrichtuniversity.nl

The peri-implantation stage in a mammalian embryo is characterized by crucial cell fate decisions by the embryonic and extra-embryonic compartments that ensure developmental progression. Around embryonic day 4.5-5.0, the primitive endoderm (PrE) differentiates into parietal endoderm (PaE), that later forms the parietal yolk sac, and visceral endoderm (VE), that will form the visceral yolk sac and part of the definitive endoderm [1]. Although embryos have been informative on the bifurcation of PaE and VE, the mechanism behind this process remains elusive. We hypothesize that the PrE differentiation into PaE and VE follows a stochastic, salt and pepper distribution, similar to the ICM differentiation into epiblast and PrE, and later migrate out with some of them undergoing an epithelial-mesenchymal transition (EMT). Recently, stem cell-based embryo models that mimic the epiblast-PrE co-development [2], recapitulate the formation of PaE and VE through chemical induction. We use this model to investigate the plasticity of these cell types and the instructive cues required to reinforce their identity. It has been shown that the signals from trophectoderm (TE) and extracellular matrix (ECM) influence differentiation and migration of PaE in natural embryos [3]. Here, we explore these parameters by screening the effect of ECM components and make use of high-throughput microwell-based platforms to identify the signaling pathway modulators and morphogenetic factors underlying this process. Understanding the implantation process of a mammalian embryo would be a developmentally relevant concept and can help in understanding the implantation failures or fertility related problems in humans.

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Abstract **#354**

GENERATION OF MESENCHYMAL STEM CELLS FOLLOWING INDUCTION OF PLURIPOTENCY IN ORAL FIBROBLASTS

Hassan Ali$_P$[1], Salwa Suliman[1], Daniela-Elena Costea[2], Helge Ræder[3], Tarig Osman[2], Kamal Mustafa[1]

[1] *Department of Clinical Dentistry, University of Bergen*

[2] *Department of Clinical Medicine, University of Bergen*

[3] *Department of Clinical Science, University of Bergen*

Corresponding author's email: Hassan.Ali@uib.no

Bone marrow is still the most common source of mesenchymal stem cells (MSC) for bone regeneration despite the limited yield of these cells by aspiration (<1%). It is thus crucial to find alternative sources for MSC and one attractive source is from induced pluripotent stem cells (IPSC). IPSC are generated through reprogramming adult cells into an embryonic like state by activating certain pluripotency genes (OCT4, SOX2, KLF4, MYC). The objective of our study was to compare the efficiency of oral and skin fibroblasts (gold standard) for reprogramming into IPSC, and the efficiency of deriving MSC from the generated IPSC.

Matched oral and skin fibroblasts were reprogrammed into IPSC via episomal plasmid transfection. The IPSC showed typical IPSC morphology (tight colonies surrounded by a light outer rim) and expressed a set of pluripotency genes (NANO-G, OCT4, SOX2). Following their characterization, oral-IPSC were differentiated into MSC (IPS-MSC) using alpha-MEM expansion media. The IPSC lost their morphology and developed a spindle shaped fibroblast morphology, typical for MSC. The resulting phenotype was characterized by expression of mRNA and protein of a standard set of MSC markers. The IPS-MSC showed in-vitro ability to differentiate into osteocyte, adipocyte and chondrocyte lineages.

The findings of our study demonstrated that oral fibroblasts can generate IPSC capable of differentiating into MSC-like cells. Oral fibroblasts were found to be comparable to skin fibroblasts in terms of IPSC generation and differentiation to IPS-MSC.

Keywords

Induced Pluripotent Stem Cells; Mesenchymal Stem Cells

Abstract **#355**

CROSS-LINKED LUNG EXTRACELLULAR MATRIX: EFFECTS ON CELL PROLIFERATION AND ANGIOGENESIS

ali shiraniP[1,2], Fatemeh Ganji[1,2], Mahtab Golmohammadi[3], Ali Samadikuchaksaraei[1,2,4]

[1] *Department of Tissue Engineering & Regenerative Medicine, School of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran*

[2] *Cellular and Molecular Research Centre, Iran University of Medical Sciences, Tehran, Iran*

[3] *Department of Genetic, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

[4] *Department of Medical Biotechnology, Iran University of Medical Sciences, Tehran, Iran*

Corresponding author's email: samadikuchaksaraei@yahoo.com

Abstract

Objective: Natural extracellular matrix (ECM) of lung tissue can be used as a component for improvement of regeneration in lung injuries. However, the mechanical prosperities of the ECM prepared after decellularization of lung tissue can affect its regeneration-inducing potential. We aimed to manipulate the mechanical properties of lung ECM by a cross-linking method while preserving its biological properties.

Methods: Rat lung tissue was decellularized using an SDS-Triton protocol and cross-linked by 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The cross-linked ECM was assessed for its potential to induce cell proliferation and angiogenesis. Cell proliferation assay was performed using human fetal mesenchymal stem cells (hFMSCs) and angiogenesis assay was performed using a chick embryo chorioallantoic membrane model. H&E and Masson's trichrome staining were also performed where appropriate.

Results: The results indicated a higher proliferation of hFMSCs on the EDC-cross-linked tissue (86.19 ± 5.973, N=3) compared to the acellular non-cross-linked tissue (67.24 ± 3.374, N=3). Also, the EDC-cross-linked tissue showed higher potential for induction of angiogenesis compared to the acellular non-cross-linked group (acelluar cross-linked group; 75.33 ± 5.044 N=5 vs acellular non-cross-linked group; 54.00 ± 4.933 N=5).

Conclusion: Lung decellularized ECM could be cross-linked by a carefully selected method without negatively affecting its biological properties.

Keywords

Cross-linking; Angiogenesis; EDC

Abstract **#356**

DESIGN AND DEVELOPMENT OF A ROBOTIC BIOPRINTER CONTROL SYSTEM AND PATH PLANNING ALGORITHM

Gabriele Maria Fortunato[P][1,2], Gabriele Rossi[1], Amedeo Franco Bonatti[1,2], Aurora De Acutis[1,2], Christian Mendoza Buenrostro[3], Giovanni Vozzi[1,2], Carmelo De Maria[1,2]

[1] *Research Centre "E. Piaggio", University of Pisa, Pisa, Italy*

[2] *Department of Information Engineering, University of Pisa, Pisa, Italy*

[3] *School of Engineering and Sciences, Tecnologico de Monterrey, Monterrey, Mexico*

Corresponding author's email: gabrielemaria.fortunato@phd.unipi.it

The aim of this work is to design a robotic bioprinting platform able to fabricate a three-dimensional structure onto irregular surfaces. Regarding the limitations of in-vitro bioprinting approach, widely used in scaffold-based tissue-engineering (handling difficulty, risk of contamination, shape not matching the defect), this bioprinter allows a direct dispensing (i.e. in-situ bioprinting) of biological material onto and into the damaged site [1-2]. The robotic platform was developed starting from the open source BCN3D-MOVEO robot [3-4]. The hardware was rebuilt to control the robot using LinuxCNC [5], the original end-effector was substituted by a syringe pump module, and joints were equipped with optical encoders. A path planning algorithm that automatically projects any generic printing pattern on an irregular surface, was developed in Matlab. For each point, the normal direction is calculated to successively constrain the end-effector orientation (always perpendicular to the surface). An inverse kinematic algorithm calculates joint angles for each position of the end-effector and a gcode file is then exported to LinuxCNC. Resolution and repeatability were estimated and preliminary in-situ bioprinting tests were carried out extruding a hydrogel onto various substrates.
The possibility of dispensing material onto irregular surfaces opens the way to a number of possibilities in the field of tissue-engineering. In this context, in-situ bioprinting could become a reality in the near future especially for the easiest accessible organs such as skin. Having a "collaborative" bioprinter, capable of assisting the surgeon during the operating phase, will allow a more precise intervention and will minimize human errors.

Keywords

in-situ bioprinting; robotic bioprinter; tissue engineering

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Abstract **#357**

MIMICKING THE DYNAMIC AND STATIC ECM: DESIGNING DOUBLE NETWORK HYDROGEL BIOINKS

Ana A Aldana$_P$[1], Francis L C Morgan[1], Sofie Houben[2], Louis Pitet[2], Lorenzo Moroni[1], Matthew B Baker[1]

[1] *Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands*

[2] *Institute for Materials Research, Hasselt University, Wetenschapspark 1, B-3590 Diepenbeek, Belgium*

Corresponding author's email: a.aldana@maastrichtuniversity.nl

Bioprinting is a leading biofabrication technique for the recreation of 3D tissue-like materials.1-3 The extracellular matrix (ECM) is a complex and dynamic structural support for cells within tissues and plays an important role in regulating cell functions.1 While significant progress has been made in bioink (cell-laden hydrogels) development, few of these materials show both stiffness and viscoelasticity comparable to native tissue, and shear-thinning properties needed for printing.4-5 Here, we developed double network (DN) hydrogel bioinks by combining a dynamic and a static cross-linked network. We investigated the combination of oxidized alginate cross-linked by Schiff-base reactions (OA, dynamic network) and polyethylene glycol diacrylate (PEGDA, static network), to develop a small library of hydrogels (OA0/2/2.5/3wt%; PEGDA0/10/15/20wt%). First, we assessed the injectability of DN and SN hydrogels, demonstrating that the polymer concentration allowed us to modulate the DN hydrogel properties, changing from soft and sticky to tough and brittle. We optimized the bioprinting parameters of hydrogels (OA2.5wt%-PEGDA0/5/10/15wt%; OA2/3wt%-PEGDA10wt%). Self-healing tests showed that hydrogels were able to heal even at 15wt% of PEGDA. A first cell-viability test corroborated their biocompatibility. Furthermore, cell seeded on the hydrogel could migrate and spread within the hydrogel, a crucial necessity for tissue maturation. So far, by combining dynamic and static networks, we developed stronger hydrogels than single networks, retaining the self-healing properties and improving the printability of DN formulations. Furthermore, both crosslinking chemistries will also allow us to incorporate bioactive molecules on both dynamic and static networks, envisioning a great potential for mimicking the dynamic ECM complexity.

Keywords

Double networks; Dynamic covalent cremistry; Bioprinting

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Abstract **#358**

ADVANCED ALLOGENEIC POROUS MATRICES FOR PERSONALIZED CELL CULTURE AND TISSUE ENGINEERING

Sara C. Santos$_P$[1], Catarina A. Custódio[1], João F. Mano[1]

[1] *Department of Chemistry, CICECO, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*

Corresponding author's email: catarinacustodio@ua.pt

Human platelet lysates (PL) play a well-known role in cell function and tissue repair[1,2]. We recently reported a bioactive PL-derivative precursor (PLMA) that can be cured upon light exposure to form hydrogels with tuneable mechanical properties able to support human-derived cells culture[2]. Animal-derived serum is widely used as supplement in cell culture, nevertheless, its use, raises many safety, scientific, and ethical concerns[1]. Following a humanized and completely xeno-free strategy we propose PL-sponges as a support for 3D cell culture in animal serum-free conditions.PLMA was prepared following a previously reported protocol[2]. Hydrogels were formed by light irradiation of PLMA solutions with photoinitiator. The human protein based sponges were produced by freeze-dry of hydrogels. Their structural and mechanical properties were assessed. To assess sponges biological performance, human adipose derived stem cells (hASCs) were seeded and cultured in medium with and without FBS supplementation. Viability tests showed that hASCs exhibit high cell viability when cultured in FBS-free medium. DNA quantification and MTS results revealed the ability of hASCs to proliferate and stay viable for 14 days even in the absence of FBS. Protein release assays results demonstrate the controlled release of proteins and growth factors involved in cell function.Our results showed that PLMA sponges are a promising platform for 3D cell culture, offering the possibility to culture cells avoiding the use of animal-derived supplements. It is also a platform that could have an autologous origin, being adequate to produce personalized matrices with no risk of cross-reactivity, immune reaction or disease transmission.

Keywords

Human platelet lysates; Xeno-free cell culture; Personalized medicine

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Abstract **#359**

MELT EXTRUSION ADDITIVE MANUFACTURED 3D SCAFFOLDS WITH ANTIMICROBIAL PROPERTIES

Carlos Mota$_P$[1], María Cámara-Torres[1], Stacy Duarte[1], Ravi Sinha[1], Ainhoa Egizabal[2], Noelia Álvarez[2], Maria Bastianini[3], Michele Sisani[3], Paolo Scopece[4], Marco Scatto[4], Alessandro Bonetto[5], Antonio Marcomini[5], Alberto Sanchez[2], Alessandro Patelli[6], Lorenzo Moroni[1]

[1] *Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue regeneration Department, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands.*

[2] *TECNALIA, Basque Research and Technology Alliance (BRTA), Mikeletegi Pasealekua 2, 20009 Donostia-San Sebastian, Spain.*

[3] *Prolabin & Tefarm S.r.l., Via dell'Acciaio, 9 06134 Perugia, Italy.*

[4] *Nadir S.r.l., Via Torino, 155/b, 30172 Venice, Italy.*

[5] *Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Dorsoduro 3246, 30172 Venice, Italy.*

[6] *Department of Physics and Astronomy, Padova University, Via Marzolo, 8, 35131 Padova, Italy.*

Corresponding author's email: c.mota@maastrichtuniversity.nl

Bone infection management after open bone fracture or implant surgery remain a challenge in the orthopedics field, and can derive into non-unions. In order to locally prevent or eradicate infections while supporting bone healing in these scenarios, 3D scaffolds with local antibiotic release properties are desired. In this study, 3D scaffolds loaded with antibiotics were processed via high temperature melt extrusion additive manufacturing (~190 °C). This was done, for the first time, by the intercalation of ciprofloxacin and gentamicin into the interlamellar spaces of the inorganic fillers magnesium aluminum layered double hydroxides (MgAl) and α-zirconium phosphates (ZrP), respectively, prior to their dispersion within a thermoplastic polymer. The inorganic fillers allowed a more sustained release of the antibiotics from the composite scaffolds, compared to no-filler systems. Moreover, it was demonstrated that the release mechanism of the antibiotics from the fillers through the polymer matrix was governed by antibiotics counter ions exchange and pH conditions in the eluent. Importantly, the manufacturing at high temperatures did not affect the antibiotics functionality, as verified by their activity against both Gram + and Gram - bacterial strains. In addition, human mesenchymal stromal cells were able to undergo osteogenic differentiation when seeded on filler-antibiotic scaffolds, as suggested by the expression of relevant osteogenic markers, such as collagen I and osteopontin, and the observation of calcium deposits in the extracellular matrix. Overall, these results suggest the possibility of improving infection management and allowing bone regeneration with these dual functionality 3D scaffolds fabricated via high temperature melt extrusion.

Abstract **#360**

MATHEMATICAL MODELLING OF REGENERATIVE ANGIOGENESIS AFTER NERVE INJURY TO INFORM THE DESIGN OF ENGINEERED REPAIR CONSTRUCTS.

Maxime Berg[P][1,2], Despoina Eleftheriadou[3,1,2], James B. Phillips[3,2], Rebecca J. Shipley[1,2]

[1] *Department of Mechanical engineering, University College London – London, WC1E 6BT*

[2] *Center for Nerve Engineering, University College London – London, WC1E 6BT*

[3] *Department of Pharmacology, University College London School of Pharmacy – London, WC1N 1AX*

Corresponding author's email: maxime.berg@ucl.ac.uk

5% of traumas result in Peripheral Nerve Injuries (PNI), affecting more than 1M people per year in Europe and the USA [1], with healthcare costs exceeding £1Bn/year in the USA alone [2]. Gold-standard treatments for large-gap PNIs use an autograft to fill the space between nerve stumps, however they also induce donor-site morbidity and often have underwhelming functional recovery [3]. Engineered Neural Tissues (EngNT [4]), made of anisotropic biomaterial hydrogels, represent a seducing alternative as their design can be tailored to support nerve repair processes.

Vascularisation of the nerve graft is key for neurite regrowth and nerve regeneration [5]. To promote vascularisation, EngNT constructs may be seeded with therapeutic cells that release vascular endothelial growth factors (VEGF) to stimulate the sprouting of new microvessels [6]. The identification of the mechanisms driving vascularisation and subsequent EngNT design optimization can be costly and time-consuming using experiments in isolation.

Here, we present a mathematical model, parameterised against in vitro data using statistical inference. The model comprises a system of coupled, non-linear, diffusion-reaction equations describing seeded cell density, oxygen and VEGF concentrations, overlaid with a discrete model for endothelial sprout growth, blood flow and intravascular transport of oxygen, representing the rich spatio-temporal dynamics between blood flow, angiogenesis, oxygen delivery, VEGF production and seeded cell survival.

This approach enables the screening of a large range of EngNT designs (e.g. seeded cell types and spatial distributions, biomaterial properties) to inform future experimental decisions along with the identification of key mechanisms driving early stages of nerve repair.

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Abstract **#361**

PLURIPOTENT STEM CELLS TOWARDS NEPHRON PROGENITOR CELLS: SYNCHRONIZATION FOR IMPROVED DIFFERENTIATION EFFICIENCY

Carlos Mota P[1], Abhishek Harichandan[1], Lorenzo Moroni[1]

[1] *Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration Department, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands.*

Corresponding author's email: c.mota@maastrichtuniversity.nl

The application of pluripotent stem cells (PSCs) as regenerative medicine strategies represent an important for patients affected by kidney disease1. Nephron progenitor cells (NPCs), the precursors of nephrons, can be generated from PSCs. Differentiation of PSCs depends on the response of the stem cells to the external induction cues. Deriving a homogeneous population of NPCs, is an essential prerequisite to generate a pure cell population. In this study, we established protocols to improve differentiation efficiency of PSCs into NPCs. The two progenitor populations, metanephric mesenchyme (MM) and ureteric bud (UB) were generated with optimized efficiency. MM and UB were combined in two-dimensions and in three-dimensions to generate kidney organoids. Results showed that an efficient synchronization of cell that respond to WNT agonist CHIR99021, thus efficiently stimulating the directed differentiation of PSCs to a homogenous population of NPCs. The NPCs homogeneity was characterized by gene expression and by immunocytochemistry and flow cytometry analysis. NPCs when synchronized gave a higher yield of renal vesicles, which eventually would give higher number of nephrons-like structures. Kidney ECM-derived hydrogel further improved the generation of renal vesicles from NPCs. Upon induction with exogenous Activin A and FGF9 in kidney derived sacrificial ECM hydrogel, NPCs successfully formed renal vesicles. Furthermore, ECM hydrogel was further combined with alginate-based materials and bioprinted showing high cell viability post-printing and up to 7 days in culture. These initial steps might allow further development of renal in vitro models for nephrotoxicity assays and for the development of future regenerative medicine strategies.

Keywords

Induced pluripotent stem cells; Extracellular matrix; bioprinting

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Abstract **#362**

ATMOSPHERIC PLASMA ASSISTED ADDITIVE MANUFACTURED 3D SCAFFOLDS FOR TISSUE ENGINEERING APPLICATIONS

Carlos Mota$_P$[1], Maria Cámara-Torres[1], Ravi Sinha[1], Paolo Scopece[2], Alessandro Patelli[3], Lorenzo Moroni[1]

[1] Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration Department, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands.

[2] Nadir S.r.l., Via Torino, 155/b, 30172 Venice, Italy.

[3] Department of Physics and Astronomy, Padova University, Via Marzolo, 8, 35131 Padova, Italy.

Corresponding author's email: c.mota@maastrichtuniversity.nl

Melt extrusion additive manufactured (ME-AM) scaffolds are ideal for tissue regeneration, due to their high porosity, customizability, and reproducible fabrication. Yet, cell-material interactions are limited on synthetic polymers used as scaffold materials, which can hinder an efficient cell adhesion, compromising the development of a functional construct. Here, a hybrid AM technology1, which combines a standard ME-AM technique with an atmospheric plasma jet, was employed to fabricate scaffolds and render their surface more bioactive in a single process. The monomers (3-Aminopropyl) trimethoxysilane (APTMS) and maleic anhydride-vinyltrimethoxysilane (MA-VTMOS) allowed the deposition of positively charged amine and negatively charged carboxyl functional groups on the scaffolds surface, respectively, while argon plasma activation led to a more unspecific surface treatment. Yet, all plasma treatments enhanced fibronectin and vitronectin cell adhesive proteins adsorption to their surface, and allowed for homogeneous human mesenchymal stromal cells (hMSCs) attachment along the scaffold cross section, compared to untreated scaffolds. Interestingly, electrostatic interactions between hMSCs and the APTMS scaffolds also allowed for homogeneous scaffold population on these scaffolds in the absence of serum. In addition, aging of the surface treatment upon scaffolds storage was also investigated, as per its clinical relevance. Notably, the scaffold wettability, as well as hMSCs attachment and proliferation were not significantly affected, compared to freshly prepared scaffolds, and osteogenic genes were expressed. Overall, the use of the hybrid AM technology enabled an efficient workflow of scaffold production and surface treatment, which proved to enhance cell-scaffolds interactions towards the creation of more functional tissue substitutes.

Keywords

Additive Manufacturing ; Plasma; Bone

References

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Abstract **#363**

DECELLULARIZED MATRIX ENRICHED METASTATIC BREAST CANCER SPHEROIDS FOR HIGH-THROUGHPUT DRUG SCREENING

Luís Ferreira[P][1], Vítor Gaspar[1], Luís Mendes[1], Iola Duarte[1], João Mano[1]
[1] *University of Aveiro - Department of Chemistry - CICECO*
Corresponding author's email: vm.gaspar@ua.pt

Decellularized extracellular matrix (dECM) is emerging as a valuable tool for generating 3D in vitro tumor models that better recapitulate tumor-stroma interactions [1]. However, the development of dECM enriched heterotypic freestanding spheroids exhibiting a controlled morphology is yet to be materialized [2]. Precisely controlling microtumors morphologic features is key to avoid aberrant cellular behavior and consequently an inaccurate evaluation of therapeutics bioperformance during preclinical screening [2]. To address this challenge, herein we employed microfiber-processed dECM for bioengineering 3D metastatic breast cancer-fibroblast models via liquid overlay technique as a bottom-up strategy to include key cellular/matrix components in reproducible spheroid-shaped microtissues. This biomimetic approach enabled the self-assembly of heterotypic dECM-3D tumor-stroma spheroids with tunable size and highly reproducible morphology. Overtime, dECM-enriched heterotypic cancer-stromal microtumors exhibited a physiomimetic necrotic core, metastatic potential, altered biochemical profiles and distinct response to different standard-of-care chemotherapeutics, recapitulating major hallmarks of the native microenvironment. Exometabolomics profiling of multicellular dECM-3D in vitro models further identified important breast cancer metabolic features including intense glycolytic activity, thus being in close correlation with in vivo breast cancer metabolism. The produced dECM-3D microtumors overcome the morphologic variability and low cell density associated with conventional cell-laden dECM hydrogel models, while providing a scalable and biomimetic testing platform that can be leveraged for high-throughput drug screening of innovative therapeutics.

Keywords
Decellularized Extracellular Matrix; Triple-Negative Breast Cancer; Tumor Spheroids

References

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Abstract **#364**

ADDITIVE MANUFACTURING OF BULK AND SURFACE GRADIENTS FOR SCAFFOLDS FOR TISSUE ENGINEERING

Carlos Mota$_P$[1], Ravi Sinha[1], Maria Cámara-Torre[1], Paolo Scopece[2], Emanuele Falzacappa[2], Alessandro Patelli[3], Lorenzo Moroni[1]

[1] Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration Department, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands.

[2] Nadir s.r.l, Venice, Italy

[3] Department of Physics and Astronomy, Padova University, Padova, Italy.

Corresponding author's email: c.mota@maastrichtuniversity.nl

Scaffolds with gradients of physico-chemical properties and controlled 3D architectures are crucial for engineering complex tissues. These can be produced using multi-material additive manufacturing (AM) techniques. However, they typically only achieve discrete gradients using separate printheads to vary compositions. Achieving continuous composition gradients, to better mimic tissues, requires material dosing and mixing controls. No such AM solution exists for most biomaterials.
A novel hybrid AM solution with dosing- and mixing-enabled, dual-material printhead and an atmospheric pressure plasma jet (APPJ) to selectively activate/coat scaffold filaments during manufacturing were combined on one platform. Continuous composition gradients in both 2D hydrogels and 3D thermoplastic scaffolds were fabricated. An improvement in mechanical properties of continuous gradients compared to discrete gradients in the 3D scaffolds. The creation of surface gradients by means of APPJ allowed a selective treatment in predesigned regions of the scaffold and the ability to selectively enhance cell adhesion was demonstrated. Bulk composition gradients have been evaluated in vitro and in vivo in a critical size long bone defect of a rabbit model. Results show a selective induction of new bone formation depending of the gradient used in comparison with controls.
The developed hybrid platform opens endless possibilities to create different gradient and non-gradient scaffolds with the possibility to fine-tune material composition and surface properties that can be valuable assets for the next generation of thermoplastic scaffolds for a plethora of TE applications.

Keywords

Additive Manufacturing ; Athmospheric plasma ; Gradient scaffolds

Abstract **#365**

FLASH: FLUORESCENTLY LABELLED SENSITIVE HYDROGEL TO MONITOR BIOSCAFFOLDS DEGRADATION DURING NEOCARTILAGE GENERATION

Carmine Onofrillo[1,2,3], Serena Duchi[1,2,3], Sam Francis[1,2], Cathal D. O'Connell[2,4], Lilith Caballero Aguilar[1,2,5], Steph Doyle[2,4], Zhilian Yue[3], Gordon Wallace[3], Peter F. Choong[1,2,6], Claudia Di Bella[1,2,6]

[1] *Department of Surgery, University of Melbourne*

[2] *Biofab3D, ACMD, St. Vincent's Hospital, Melbounre*

[3] *ARC, IPRI, University of Wollongong*

[4] *Discipline of Electrical and Biomedical Engineering, School of Engineering, RMIT University, Melbourne*

[5] *Faculty of Science, Engineering and Technology, Swinburne University of Technology, Melbourne*

[6] *Department of Orthopaedics, St Vincent's Hospital Melbourne*

Corresponding author's email: carmine.onofrillo@unimelb.edu.au

The use of bioscaffolds made of photocrosslinkable hydrogels and stem cells is widely endorsed in the field of cartilage repair (1, 2). Cell mediated degradation of the implanted bioscaffolds is critical for their therapeutic efficacy (3). Thus, tuning the balance between biodegradability and deposition of a distinct new extracellular matrix is key to control the chondrogenic potential.

In this study we generated FLASH, a Fluorescently LAbelled Sensitive Hydrogel to correlate the degradation of a bioscaffold with neocartilage formation. Gelatine Methacryloyl (GelMA) was covalently bound to the FITC fluorophore to generate FLASH and bioscaffolds were produced by casting different concentrations of FLASH GelMA, with and without human adipose-derived stem cells (hADSCs) undergoing chondrogenesis. The loss of fluorescence from FLASH bioscaffolds was correlated with changes in mechanical properties, expression of chondrogenic markers and accumulation of a cartilaginous extracellular matrix. The ability of the system to monitor the bioscaffold degradability during chondrogenesis was evaluated in vitro, in a human ex vivo model of cartilage repair and in a full chondral defect in vivo rabbit model. Regardless of the experimental models used, the extracellular matrix production within the hydrogel correlates with the fluorescence loss profiles, demonstrating that FLASH is a sensitive tool to monitor photocrosslinkable hydrogels in tissue engineered constructs. This study represents a step towards the generation of a high throughput monitoring system to evaluate de novo cartilage generation in tissue engineering therapies.

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Abstract **#366**

DESIGN AND OPTIMISATION OF A NOVEL FLUIDISED BED BIOREACTOR FOR THE EXPANSION OF ERYTHROID PROGENITOR CELLS

David Phillips[P][1], Parimala Shivaprasad[1], Marianne Ellis[1], Sandhya Moise[1]

[1] *Centre for Integrated Bioprocessing Research (CIBR), Dept. of Chemical Engineering, University of Bath, Bath, United Kingdom*

Corresponding author's email: dp569@bath.ac.uk

More than 117 million units of blood are transfused worldwide, annually1. These units are sourced from donors, however whilst demand is rising, donor numbers are falling 2. In the UK alone, ~400 new donors are required daily to maintain provision. Compounding these shortfalls are the risks of infectious blood-borne diseases1, the scarcity of rare blood-group donors, and the extensive typing required to limit immunogenicity in the chronically transfused3. Safe, reliable, and cost-effective sources of blood are therefore required, particularly for its erythrocyte component. The recent creation of the first erythroid cell-line (BEL-A) provides a novel, scalable source for in vitro production of erythrocytes without need of donors2. However, low yields and high costs pose challenges for clinical translation.

Our research focuses on designing and optimising a novel fluidised bed bioreactor (FBB) to scale-up BEL-A cell production to therapeutically-relevant levels. When fluidised within the FBB, each cell remains individually suspended in media, achieving superior mass transfer under low-shear compared to traditional systems. Hence, when optimised, FBBs present attractive large-scale cellular manufacturing platforms. Following successful culture of BEL-As in our proof-of-concept studies, our current focus is identifying FBB design and operating conditions which improve yields. In addressing this, we have adopted a design of experiments approach to analyse the individual and interactive effects of these conditions. Furthermore, we are performing stoichiometric analysis of BEL-A culture to define media recirculation capacity. Results of this study are a major step towards efficiently producing erythrocytes at clinical-scale and establishing a platform technology for cell-therapy manufacture.

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Abstract **#368**

DEVELOPMENT OF SELF-REPLICATING MRNA VACCINE AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

Yi-Hao Chang[P][1], Ren-Li Lin[1], Yu-Chen Hu[1], Chin-Yu Lin[2]

[1] *Department of Chemical Engineering, National Tsing Hua University, Taiwan*

[2] *Institute of Drug Development, China Medical University, Taiwan*

Corresponding author's email: taros_yihau@hotmail.com

Porcine reproductive and respiratory syndrome (PRRS) is a serious infectious swine disease, causing major economic losses to the world-wide pig industry. PRRS manifests differently in pigs of all ages but primarily causes late-term abortions and stillbirths in sows and respiratory disease in piglets. The causative agent of the disease is the positive-strand RNA PRRS virus (PRRSV). Therefore, it is necessary to develop a new type of PRRSV vaccine that can efficiently induce specific immune responses quickly in order to produce cross protection. In this study, we aim to develop a new mRNA-based PRRSV vaccine by self-replicating VEE-RNA replicon that expresses the antigens of PRRSV by using in-vitro transcription. Primary test for PK-15 (Porcine Kidney cell line) confirmed that VEE replicon can persistently express the antigens for 4 weeks. Moreover, we demonstrate that a single low dose intradermal immunization with lipid-nanoparticle-encapsulated nucleoside modified mRNA (mRNA–LNP) encoding glycoproteins (GP5) of PRRSV VR-2332 elicited persistent neutralizing antibody responses in mice. Compared to traditional attenuated vaccine or modified live virus (MLV), mRNA vaccine can induce specific immune responses earlier and is safer to use.

Keywords

PRRSv; mRNA vaccine

Abstract **#369**

DEVELOPMENT OF NOVEL 3D MODELS FOR DRUG SCREENING IN MESOTHELIOMA

Yiwei Wang[P][1], Antony Linton[2], Kedong Song[3], Kenneth Lee[4], Ben Johnson[2], Ling Zhuang[2], Yuen Yee Cheng[2]

[1] Burns Research and Reconstructive Surgery Group, ANZAC Research Institute, Faculty of Medicine and Health, The University of Sydney, NSW, Australia

[2] Asbestos Diseases Research Institute, Faculty of Medicine and Health, The University of Sydney, NSW, Australia

[3] State Key Laboratory of Fine Chemicals, Dalian R&D Centre for Stem cell and Tissue Engineering, Dalian University of Technology, Dalian, China

[4] Anatomical Pathology, Concord Hospital, The University of Sydney, NSW, Australia

Corresponding author's email: yiweiwang@anzac.edu.au

Background:

Cancer drug screening studies rely heavily on the use of conventional two-dimensional (2D) cell culture systems, which has significant limitations in its ability to model tumour cell morphology, signalling, gene expression, architectural features and response to oxygen. Therefore, development of 3D cell culture models has attracted growing interest in cancer research. Malignant pleural mesothelioma (MPM) is an aggressive asbestos-related thoracic cancer. Chemotherapy is an important palliative treatment option but almost every patient will be confronted with recurrence of disease and drug resistance.

Methods:

MPM tumour spheroids and MPM cells grown in decelluarised porcine lung scaffolds were developed and examined. MicroRNA gene expression profiling was investigated, while hypoxia (Hif1a, one of the major hallmarks of solid tumours) was analysed using immunohistochemistry. Drug cytotoxicity of cisplatin and gemcitabine was investigated.

Results:

MPM 3D spheroids and MPM-scaffolds represent mini-tumour models as indicated by the visualization of cell junctions under TEM. Tumour suppressor biomarkers we analysed showed down-regulation of mRNA expression level compared with cells in 2D. MPM spheroid showed up-regulation of genes that contribute to drug resistance such as Hif1a and YAP1. The 3D models exhibited a greater resistance to cisplatin and gemcitabine compared to 2D cultures. Immunofluorescence studies showed a gradient of hypoxia from the centre of the spheroids or scaffolds where high Hif1a expression is observed.

Conclusions:

3D cells grown with the newly optimised method provide a more realistic mimic of clinical drug response to MPM, as evidenced by the greater resistance of MPM cells to cisplatin and gemcitabine.

Keywords

Drug screening; 3D model; Mesothelioma

Abstract **#370**

RETHINKING OF ANTIMICROBIAL BIOMATERIAL DESIGNS - IMPLICATIONS FROM AN IN VITRO COCULTURE STUDY

Phong Tran$_P$[1]

[1] *Queensland University of Technology*

Corresponding author's email: phong.tran@qut.edu.au

There is significant interest in anti-infective biomaterials that have a dual property of promoting tissue integration and inhibiting bacterial adhesion to prevent device-associated infection. However, it is also known that bacteria such as S. aureus have abundant surface pathogen-associated molecular patterns can readily interact with mammalian cells such as osteoblasts. Using tissue culture plastic, we found that S. aureus were electrostatically repelled from this surface and that when they were inoculated onto this surface pre-seeded with MC3T3-E1, they formed aggregates that adhered strongly to these osteoblastic cells. Importantly, the bacteria in the aggregates were more resistant to gentamicin compared to those in bacterial monoculture. This finding thus suggested that the bacteria repelled from such a surface might end up establishing a biofilm-like community on adjacent mammalian cells or tissue and that anti-infective biomaterials should actively kill bacteria.

Keywords

bacterial; biofilm; infection

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Abstract **#371**

NUMERICAL AND EXPERIMENTAL STUDY OF HAEMODYNAMICS AND WALL COMPLIANCE IN THE ANASTOMOSIS OF SMALL-DIAMETER VASCULAR GRAFTS WITH PATIENT-SPECIFIC GEOMETRIES FOR TRANSLATION TO TISSUE ENGINEERING

Sabrina Schoenborn[P][1], Mia Woodruff[1], Mark Allenby[1]
[1] *Queensland University of Technology*
Corresponding author's email: s.schoenborn@uq.edu.au

The primary cause of mid-term occlusive failure in small-diameter vascular bypass grafts is the development of intimal hyperplasia driven by mechanical compliance mismatch [1-3]. While current tissue-engineered vascular grafts (TEVGs) achieve physiological tubular compliance, (pre)clinical outcomes remain limited due to the lack of compliant and biodegradable TEVG anastomosis solutions [4-6]. To address this unmet need, computational and experimental models have been developed to design and test anastomosis solutions for TEVGs.This numerical study is based on a three-dimensional computer model of an artery-graft anastomosis with investigation of wall compliance being facilitated via a two-way fluid-structure interaction (FSI) model. The wall deformations and fluid dynamics around the anastomosis site are investigated in patient-specific geometries derived from medical imaging data for different anastomosis solutions with graft types of varying compliance. The numerical results are then experimentally validated in a vascular testing rig using arterial phantoms to create anastomoses.We have developed FSI simulations of blood flow within a compliant artery for an idealised patient geometry which shows mechanical compliance consistent with published literature. We will create idealised and patient-segmented peripheral artery simulations of different anastomosis techniques to investigate the effect of anastomotic and tubular compliance on shear stress and fluid flow profiles. Using these numerical results, we will design, 3D print, and experimentally validate TEVG anastomosis devices for improved anastomotic compliance.A numerical FSI model capturing the complex relationship between anastomotic wall compliance and haemodynamics can be an important tool in the design and evaluation of patient-specific graft-anastomosis solutions enabling TEVG translation.

Keywords
Anastomosis; Fluid-Structure Interaction; Tissue-Engineered Vascular Grafts

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Abstract **#372**

MICROENVIRONMENTAL MODIFICATION BY THROMBOMODULIN AND SURROUNDING CELLS FOR PNS REGENERATION

Chia-Ching (Josh) Wu[P][1], Tzu-Chieh Huang[1], Chia-Wei Huang[1]
[1] *National Cheng Kung University*
Corresponding author's email: joshccwu@mail.ncku.edu.tw

Peripheral nerve regeneration is usually slow and incomplete. Currently, inflammation has been identified as a therapeutic target of peripheral nerve regeneration. We are interested to evaluate the novel potential of thrombomodulin (TM) in promoting peripheral nerve regeneration by regulating macrophage polarization. We found that TM can facilitate M1 switching toward M2, indicated by reduced inflammatory cytokine levels and a concomitant increase in M2 markers, through a STAT6-PPARγ pathway. The conditional medium derived from TM-treated M1 exhibited a mild inflammatory induction capacity as compared to that of M1. When testing in nerve transcended model, TM treatment led to better nerve regeneration and preserved effector muscle mass. We also observed evident M1 to M2 transition in TM-treated nerve. TM appears to be a drug for the promotion and modulation of functional recovery in peripheral nerves by enhancing M1 to M2 transition. Myelination is an important process during functional nerve regeneration. We also discovered the Schwann cells (SCs) can be derived from the formation of adipose-derived stem cells (ASCs) into spheres on a chitosan-coated microenvironment with the supplement of FGF9 peptide (FGF9-NLCs). The FGFR2 and FGFR4 were significantly increased during NLCs induction. The FGF9 treated FGF9-NLCs spheres became smaller and changed into SCs with expressing of S100β and GFAP. Transplanted FGF9-NLCs participated in myelin sheath formation to enhance axonal regrowth. Taken together, we found several drugs that can target on microenvironmental and therapeutic cell modulations to promote nerve regeneration and functionalization.

Keywords
Adipose Stem Cell; PNS; Macrophage

Abstract **#373**

POINT-OF-CARE CELL-BASED STRATEGY FOR TREATING LARGE CRANIOFACIAL BONE DEFECTS

Srujan Singh$_P$[1,2,3], Ethan Nyberg[2,3], Aine O'Sullivan[2,3], Ashley Farris[2,3], Nicholas Zhang[2,3], Alexandra Rindone[2,3], Eszter Mihaly[2,3], Emma Whitehead[2,3], Catherine Takawira[4], Joseph Lopez[5], Mandi Lopez[4], Michael Grant[5,6], Warren Grayson[2,3]

[1] *Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD*

[2] *Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD*

[3] *Translational Tissue Engineering Center, Johns Hopkins University, Baltimore, MD*

[4] *Department of Veterinary Clinical Sciences, Lousiana State University, Baton Rouge, LA*

[5] *Department of Plastic & Reconstructive Surgery, Johns Hopkins University, Baltimore, MD*

[6] *R Adams Cowley Shock Trauma Center, University of Maryland, Baltimore, MD*

Corresponding author's email: ssingh95@jhu.edu

Critical-sized craniofacial bone defects (CCDs) arising from trauma, congenital abnormalities, tumor resections, etc. often require surgical reconstruction to restore aesthetics and functionality. Autografts still remain the gold standard treatment, however, suffer from challenges like donor site morbidity, limited tissue availability and anatomical mismatch. To address this, we have developed a point-of-care (POC) cell-based strategy for treatment of clinically relevant large craniofacial bone defects and demonstrated its feasibility and efficacy using a pre-clinical porcine model.

2 cm full thickness, segmental defects were created bilaterally in the zygomatic bones of skeletally mature, castrated, male Yucatan pigs to mimic CCDs. The defects were treated with custom-designed, 3D-printed hybrid scaffolds comprised of polycaprolactone (PCL) and decellularized bone (DCB). Autologous stromal vascular fraction (SVF) harvested from subcutaneous porcine fat were seeded into the scaffolds. There were three experimental groups: (i) Empty (untreated), (ii) Acellular (scaffold only), and (iii) Cellular (scaffold + SVF). CT scans were performed post operatively and at 6, 12, 24 and 52 weeks to evaluate new bone formation. On reaching endpoint zygomas were harvested and biomechanical testing was performed to assess osseo-integration of scaffolds.

After 52 weeks, we have found non-union of defects in the empty group. Acellular and cellular groups exhibited robust bone regeneration with mean bone to defect volume (BV/DV) calculated to be 0.64±0.09 and 0.65±0.08 respectively with no statistically significant difference. Interestingly, mean fracture torque for zygomas from cellular group (7.1±2.3 Nm) was found out to be higher and statistically significant as compared to acellular group (2.8±0.2 Nm).

Abstract **#374**

CELL SUICIDE-BASED FUNCTIONAL CONTROL OF GENETICALLY MODIFIED CELLS FOR CELL-BASED GENE THERAPY

Kosuke Kusamori[P][1], Mari Tsujimura[1], Makiya Nishikawa[1]

[1] *Tokyo University of Science*

Corresponding author's email: kusamori@rs.tus.ac.jp

Transplantation of genetically modified cells that continuously produce a specific protein is expected to be an excellent cell-based gene therapy approach. However, an additional strategy is required to control the function of the transplanted cells for effective and safe cell-based gene therapy. We have focused on drug-induced cell death, in which the cells transduced with a suicide gene undergo cell death by addition of an inactive prodrug. The herpes simplex virus thymidine kinase (HSVtk) gene is the most famous and frequently used suicide gene, which induces cell death in the cells expressing it in combination with ganciclovir (GCV). In a previous study, we selected this combination and developed the cell regulation system, in which the proliferation and function of transplanted cells can be controlled as required by adjusting the GCV concentration. In this study, we tried to apply this cell regulation system to cell-based gene therapy to control the transgene expression level and profile. To achieve this, mouse mesenchymal stem C3H10T1/2 cells and interferon-gamma (IFN-g) gene, an antitumor cytokine, were selected, and C3H10T1/2/HSVtk/IFN-g cells were established by transfecting the cells with HSVtk gene and IFN-g gene. C3H10T1/2/HSVtk/IFN-g cells released IFN-g and underwent cell death by addition of GCV. C3H10T1/2/HSVtk/IFN-g cells significantly inhibited the proliferation of mouse adenocarcinoma colon26 cells in cell culture and retarded tumor growth in colon26 tumor-bearing mice. Moreover, transplanted C3H10T1/2/HSVtk cells were eliminated by GCV administration. These results indicate that we have succeeded in controlling the function of the transplanted cells using cell suicide-based cell regulation system.

Keywords

Cell-based gene therapy; Functional control; Suicide gene

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Abstract **#375**

3D PRINTING OF SILK-BIOCERAMIC NANOCOMPOSITES FOR THE REGENERATION OF INNERVATED AND VASCULARIZED BONE TISSUE

Vincent Fitzpatrick[P][1], David Kaplan[1]

[1] *Tufts University*

Corresponding author's email: vincent.fitzpatrick@tufts.edu

Bone is a dynamic, innervated and vascularized tissue with the inherent ability to regenerate after injury. Nevertheless, non-union fractures remain a major issue in orthopedic surgery. Autografts and allografts are the gold standard for repair but can be associated to donor site morbidity, donor material availability, and increased operative times. Alternative biomaterial-based solutions are promising, including synthetic bone fillers. However, these fillers do not allow refined control of graft geometry, and seldom offer adequate porosity for rapid cell colonization, efficient nutrient exchange, or implant degradation. Further, these approaches usually do not actively promote bone growth, nor account for the complexity of bone, and in particular the neurovascular network.

We developed a silk-ceramic bioink to 3D print porous constructs for bone regeneration. The resulting material was mechanically close to bone, promoting osseointegration. These structures were biocompatible, their shape well-controlled even for complex geometries, and their porosity could be precisely tuned for efficient osteogenesis.

Our constructs supported osteoblastic differentiation in vitro, and a rapid cell response and efficient neoosteogenesis when combined with osteoinductive factors (BMP-2). We further loaded this bioink with angiogenic and neurotrophic morphogens (VEGF and NGF), and precisely patterned these biomolecules, promoting the migration, organization and differentiation of human umbilical vein endothelial cells and human induced neural stem cells, respectively. Coculture conditions showed that the multiple morphogens and cell types promoted the osteogenic differentiation of human mesenchymal stem cells.

Finally, our bioink could be loaded with antibiotics, providing an encouraging solution for the prophylactic treatment of osteomyelitis in non-union fractures.

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Abstract **#376**

DEVELOPMENT OF A CELL-DERIVED EXTRACELLULAR MATRIX (ECM) HYBRID BIOMATERIAL TAILORED TO EXHIBIT SUPERIOR BIOACTIVITY FOR THERAPEUTIC ANGIOGENESIS.

Anna Maria Blocki[P][1,2,3], Marisa Assunção[1,2], Thomas Später[4]

[1] *Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong*

[2] *School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong*

[3] *Department of Orthopaedics & Traumatology, Faculty of Medicine, The Chinese University of Hong Kong*

[4] *Institute for Clinical and Experimental Surgery, University of Saarland, Germany*

Corresponding author's email: anna.blocki@cuhk.edu.hk

As cell-derived extracellular matrix (CDM) partially recapitulates native tissue niches, it enables the production of microenvironments with customizable properties. These can be processed into biomaterials with sustainable bioactivity and sufficient biological complexity to guide intricate tissue processes such as healing and regeneration. Nonetheless, low amounts of CDM that can be sourced in vitro, as well as the limited understanding of ECM biology are major limiting factors in the development and clinical application.

In order to engineer a biomaterial with enhanced pro-angiogenic properties, we have integrated the pro-regenerative ECM of mesenchymal stem cells (MSCs) with the heparan sulfate-mimetic dextran sulfate (DxS).

DxS aggregated and co-precipitated various ECM components during cell-driven ECM assembly, leading to an accumulation of a rich CDM with unique mechanical and topographical properties [1]. This DxS-ECM facilitated endothelial sprouting in vitro, significantly exceeding the effect of unmodified MSC-derived ECM (cECM) and collagen I. Implantation of these CDMs into full-thickness skin defects within mouse dorsal skinfold chambers demonstrated that DxS-ECM significantly enhanced functional re-vascularization and accelerated wound closure, as compared to cECM and collagen I.

Investigation of the molecular constitution revealed that co-precipitation of CDM with DxS resulted in an ECM with a qualitatively and quantitively different composition. Proteomics analysis further identified potential new players responsible for its mechanism of action.

In conclusion, we have created a CDM hybrid biomaterial with superior pro-angiogenic properties in vitro and in vivo. This biomaterial has the potential to serve as a highly effective treatment for ischemic diseases.

Keywords

extracellular matrix engineering; wound healing; heparan sulfate mimetic

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Abstract **#379**

PROGENITOR CELLS DERIVED FROM HEALTHY AND OSTEOARTHRITIC HUMAN CARTILAGE SHOW POTENTIAL FOR CARTILAGE TISSUE ENGINEERING

Margot Rikkers[P][1], Jasmijn Korpershoek[1], Riccardo Levato[1,2], Jos Malda[1,2], Lucienne Vonk[1,3]

[1] *Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands*

[2] *Department of Equine Sciences, Faculty of Veterinary Sciences, Utrecht University, Utrecht, The Netherlands*

[3] *CO.DON AG, Teltow, Germany*

Corresponding author's email: m.rikkers-2@umcutrecht.nl

Articular cartilage-derived progenitor cells (ACPCs) offer new interesting avenues as a cell source to use for cartilage tissue engineering. Unlike mesenchymal stromal cells (MSCs), ACPCs do not have the tendency to differentiate into the hypertrophic lineage. This study aims to investiage the potential of ACPCs for cartilage tissue engineering and clinical application.

Cells were isolated from healthy (n=6, age 46-49, mean age 48) and osteoarthritic (n=6, age 41-82, mean age 62) human knee cartilage. ACPCs were isolated from the total cell population by clonal growth after differential adhesion to fibronectin. Cells were characterized by cell surface marker expression, growth kinetics, colony-forming efficiency, and multilineage differentiation. ACPCs were compared to full-depth chondrocytes derived from the same donors. Next, ACPCs were cultured in 3D pellets to investigate neo-cartilage formation.

Healthy and osteoarthritic ACPCs were successfully isolated and differentiated into the adipogenic and chondrogenic lineage, but failed to produce calcified matrix when exposed to osteogenic induction media. Both ACPC populations, as well as full-depth chondrocytes met the criteria for cell surface marker expression to define MSCs as determined by flow cytometry. Cartilage-like matrix production was successful in ACPC pellet cultures.

In conclusion, this study provides further insight into a progenitor cell population which is present in both healthy and osteoarthritic human articular cartilage. The populations show similarities to MSCs, yet the ACPCs did not produce calcified matrix under well-established osteogenic and mineralization culture conditions. Furthermore, ACPCs show potential for cartilage tissue engineering and possibly for clinical application, as a promising alternative to MSCs.

Abstract **#380**

TOWARDS PATIENT SPECIFIC VASCULAR DEVICES: MULTI-SCALE ADDITIVE MANUFACTURING OF POLY(Ɛ-CAPROLACTONE) MECHANICALLY ENHANCED WITH GRAPHENE OXIDE

Trent Brooks-Richards[P1], Naomi Paxton[1], Matthew Lanaro[1], Arixin Bo[1], Lukas Michalek[1], Nathan Boase[1], Mark Allenby[1], Maria Woodruff[1]

[1] *Queensland University of Technology*

Corresponding author's email: trent.brooksrichards@qut.edu.au

Cardiovascular disease is the leading cause of death and disability worldwide. Bioresorbable vascular stents are a highly anticipated transition from metallic stents, showing potential to negate latent complications while permitting restoration of vascular function [1]. Recent results with bioresorbable stents have seen little success largely due to a need for materials with improved mechanical and degradation characteristics [2].

Here, we show a preliminary comparative investigation into the use of graphene oxide (GO) to mechanically reinforce medical-grade poly(ε-caprolactone) (PCL) fabricated using both fused deposition modelling and melt electrowriting (MEW) toward use in patient-specific bioresorbable stent technology. The application of 3D scanning, modelling and printing opens avenues for manufacture of patient-specific treatments for complex lesions [3].

For the development of 3D printed tubular structures, medical grade PCL (Purasob® PC 12, Corbion Purac, Netherlands) was combined with graphene oxide at 0.1, 0.5, 1.0 and 5.0 wt%. GO was characterised with transmission electron spectroscopy, Raman, EDSX and AFM. A rheology-based model was used to calculate the extrusion parameters based on composite viscosity. Composites were loaded into a custom MEW printer and extruded through a 21 gauge needle (Nordson, Ohio, USA) at 90°C, with a voltage difference of 4.5 kV applied from the needle tip to the mandrel at a 5 mm working distance. Gcode was generated in Repetier Host 3D printing program (Hot-World GmbH & Co. KG, Willich, Germany). Pore angles of 20°, 60°, 90° and 120° were printed with five layers with a 4 mm pore area.

Keywords

Composite biomaterials; Graphene Oxide; Vascular devices

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Abstract **#383**

GENERATION OF TWO HUMAN INDUCED PLURIPOTENT STEM CELL LINES DERIVED FROM TWO JUVENILE NEPHRONOPHTHISIS PATIENTS WITH NPHP1 DELETION

Yutaka Arai[P][1,2], Dorian Luijkx[1], Mami Matsuo Takasaki[1], Yasuko Hemmi[1], Tamami Wakabayashi[1], Jun Inoue[3], Michiya Noguchi[3], Yukio Nakamura[3], Keisuke Sugimoto[4], Tsukasa Takemura[5], Keisuke Okita[6], Kenji Osafune[7], Minoru Takasato[8], Tadayoshi Hayata[2], Yohei Hayashi[1]

[1] IPS Cell Advanced Characterization and Development Team, BioResource Research Center, RIKEN, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

[2] Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences and Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan

[3] Cell Engineering Division, BioResource Research Center, RIKEN, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

[4] Department of Pediatrics, Kindai University Faculty of Medicine, 377-2 Ohno-higashi, Osaka-Sayama 589-8511, Japan

[5] Kushimoto Municipality hospital, 691-7, Sangodai, Kushimoto-cho, Higashimuro-gun, Wakayama, 649-3510, Japan

[6] Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

[7] Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application (CiRA), Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606- 8507, Japan

[8] Laboratory for Human Organogenesis, RIKEN Center for Biosystems Dynamics Research, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

Corresponding author's email: yutaka.arai@riken.jp

Juvenile nephronophthisis is an inherited renal ciliopathy, causing cystic kidney disease, renal fibrosis, and end-stage renal failure in children and young adult. Mutations in NPHP1 gene encoding nephrocystin-1 protein have been identified as the most frequent responsible gene and cause the formation of cysts in renal medulla. The disease mechanism of juvenile nephronophthisis is little known yet, so no effective medicines exist even today. Because Nphp1 mutant mice failed to recapitulate renal manifestations of nephronophthisis (Jiang et al., 2008), faithful disease models are required to dissect molecular pathogenesis. To develop disease models, which can lead to develop effective therapies for juvenile nephronophthisis, we established disease-specific hiPSC lines from juvenile nephronophthisis patients, carrying NPHP1 mutations. Juvenile nephronophthisis patient-derived hiPSC lines were generated from peripheral blood mononuclear cells of each juvenile nephronophthisis patient using episomal vectors carrying reprogramming factors under feeder-free culture conditions. Generated hiPSC lines formed typical human embryonic stem cell-like colonies in feeder-free culture conditions, and also showed the expression of self-renewal markers and carried a large deletion in NPHP1 gene. Furthermore, both juvenile nephronophthisis-specific hiPSC lines formed embryoid bodies in vitro and showed the pluripotency for three germ layers. Since the molecular pathogenesis caused by NPHP1 dysfunction remains unclear, these cell resources provide useful tools to establish disease models and to develop new therapies for juvenile nephronophthisis.

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Abstract **#384**

ENGINEERING OF INJECTABLE MULTIFUNCTIONAL POLYURETHANE-BASED HYDROGELS FOR THE ADVANCED TREATMENT OF HARD-TO-CLOSE WOUNDS

Rossella Laurano[P][1], Monica Boffito[1], Alice Zoso[1], Valeria Chiono[1], Gianluca Ciardelli[1]
[1] *Politecnico di Torino - Department of Mechanical and Aerospace Engineering*
Corresponding author's email: rossella.laurano@polito.it

To improve the hard-to-close wound treatment effectiveness and minimize side-effects on not-target tissues, the design of smart drug carriers locally releasing their payload in a controlled way is attracting widespread interest. The work was aimed at designing a multi-functional injectable delivery platform for wound treatment, overcoming the main limitations of traditional wound dressings, allowing shape-adaptability to defects through rapid post-injection gelation and controlled drug release kinetics. The aims were achieved by exploiting the versatile chemistry of polyurethane (PU) for the synthesis of multi-functional polymers and taking advantage of the alkaline characteristics of chronic wounds exudates when colonized by bacteria.
Specifically, PU amphiphilicity was ensured by selecting Poloxamer®407 as macrodiol; alkaline pH-responsiveness was introduced by plasma-treating PU powders to expose carboxyl groups (i.e., 5.3×10E18±0.6×10E18 units/g of polymer) while preserving PU molecular weight (Mn: 29±1 kDa) and hydrogel thermo-responsiveness (gelation within 7 minutes at 37 °C for 15% w/V concentrated systems). Hydrogel thermo-sensitivity was exploited to: (1) encapsulate drugs (e.g., Ibuprofen, 1 mg/mL) in the sol state, (2) tune hydrogel viscosity for its easy injection up to room temperature through G18 needles, (3) localize payload release, and (4) perfectly fill the wound cavity. On the other hand, hydrogel pH-responsiveness was explored to enhance drug release in the presence of alkaline exudate. Hydrogel was biocompatible according to ISO10993, and able to quickly change its internal pH (i.e., pHchange_5min=3.76 vs. 2) and release high drug amounts when incubated with alkaline buffer compared to an acid milieu (approx. 60% vs. 8% within 1 h).

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Abstract **#385**

COLLAGEN TYPE 1 AND ENDOTHELIAL CELL CO-CULTURE IMPROVES B-CELL FUNCTIONALITY AND RESCUES PANCREATIC ECM PROTEIN EXPRESSION IN A TISSUE-ENGINEERED POST-TRANSPLANTATION MODEL

Max Urbanczyk[P][1], Aline Zbinden[1], Shannon L. Layland[1], Lucas Becker[1,2], Julia Marzi[1,2,3], Mariella Bosch[1], Peter Loskill[1,4], Garry P. Duffy[5], Katja Schenke-Layland[1,3,6]

[1] Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany

[2] Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University Tübingen, Germany

[3] NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany d Fraunhofer IGB, Stuttgart, Germany

[4] Fraunhofer IGB, Stuttgart, Germany

[5] Anatomy & Regenerative Medicine Institute, School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Ireland

[6] Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Corresponding author's email: max.urbanczyk@med.uni-tuebingen.de

Biomaterials and biomaterial functionalization are promising methods to support pancreatic islet viability post-transplantation in an effort to reduce insulin dependence for patients afflicted with diabetes mellitus type 1. Assessing how endogenously expressed pancreatic ECM proteins are affected by post-transplant-like hypoxic conditions may provide significant insights towards the development of tissue-engineered therapeutic strategies to positively influence β-cell survival, proliferation and functionality.

To better understand which ECM proteins may play a role in β-cell homeostasis, we investigated the expression of three relevant groups of pancreatic ECM proteins in human native tissue, including BM proteins, proteoglycans and fibril-forming proteins. In an in vitro hypoxia model, we identified that ECM proteins were differently affected by hypoxic conditions, contributing to an overall loss of β-cell functionality. The use of a COL1 hydrogel as carrier material mitigated the hypoxic impact on proteoglycans as well as fibril-forming protein expression, supporting β-cell functionality in hypoxia. The addition of endothelial cells (ECs) into the COL1 hydrogel improved β-cell response as well as the expression of relevant BM proteins. Our data show that β-cells benefit from a microenvironment composed of structure-providing COL1 with incorporation of paracrine signaling ECs to withstand the harsh conditions of hypoxia. Such hydrogels support β-cell survival and can serve as initial source of ECM proteins to allow cell engraftment while preserving cell functionality post-transplantation.

Keywords

β-cells; hypoxia; extracellular matrix

Abstract **#387**

FUNCTIONALIZABLE AND MECHANICALLY TUNABLE BIOMATERIAL FOR EMB3D PRINTING OF SPATIOTEMPORALLY CONTROLLABLE PRE-VASCULARIZED TISSUES

Malin Becker[P][1], Maik Schot[1], Jeroen Leijten[1]
[1] *Department of Developmental BioEngineering, TechMed Centre, University of Twente, The Netherlands*
Corresponding author's email: m.l.becker@utwente.nl

INTRODUCTION: Embedded 3D (EMB3D) printing is a promising approach to engineer complex tissues such as patterned or pre-vascularized tissue constructs[1,2]. However, the resulting tissue constructs are often mechanically weak, unable to form mechanical or chemical gradients, and lack on-demand tunability. Here, we report on the use of a dually crosslinkable dextran-based hydrogel as a bath for EMB3D printing, allowing for local on-demand functionalization and formation of spatial mechanical gradients.
METHODS: Dextran was functionalized with tyramine and biotin moieties to create a dually crosslinkable polymer[3]. Physically crosslinked embedding baths were created via biotin/avidin protein/ligand interaction. A gelatin based sacrificial bioink was extruded into the hydrogel using an Inkredible+ 3D printer. Covalent enzymatic or photo-initiated crosslinking of the printing bath was used to create mechanically robust tissues. The tissue's biotin moieties were subsequently used for on-demand mechanical or biochemical functionalization of the bulk and/or the channel surfaces.
RESULTS & DISCUSSION: Rheological characterization of the physically crosslinked bath revealed shear-thinning and self-healing properties that were highly suitable for EMB3D printing. Covalent crosslinking resulted in a 3-fold increase of the storage modulus of the hydrogels, which enabled the stabilization of printed channel networks, while diffusion of crosslinking agents from the ink resulted in controllable stiffness gradients in the bulk. Furthermore, the ink/bath interface allowed for one-step functionalization by loading the ink with biotin-coupled cell bioinstructive moieties.
CONCLUSION: We report on a novel and dually crosslinkable hydrogel suitable for EMB3D printing, which offers mechanical stability, mechanical tunability, with on-demand biochemical functionalization of pre-vascularized engineered tissues.

Keywords
EMB3D; vascularization; dual crosslink

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Abstract **#388**

LARGE-SCALE PRODUCTION OF SELF-DIFFERENTIATING ENGINEERED MUSCLES WITH ADVANCED MATURATION AND PROVED FUNCTIONALITY USING AN OPTIMIZED BIOPRINTABLE MATERIAL.

Andrea Garcia Lizarribar$_P$[1,2], Aranzazu Villasante[1,3], Josep Samitier[1,2,3]

[1] *Nanobioengineering group, Institute for Bioengineering of Catalonia (IBEC-BIST). 08028 Barcelona, Spain.*

[2] *Centro de Investigación Biomédica en Red (CIBER-BBN). 28029 Madrid, Spain.*

[3] *Department of Electronic and Biomedical Engineering, University of Barcelona. 08028 Barcelona, Spain.*

Corresponding author's email: agarcial@ibecbarcelona.eu

Skeletal muscle is in charge of body locomotion and homeostasis. It is one of the most important energy reservoirs and plays an important role in several chronic diseases as diabetes and cancer. Drug discovery for muscle disease treatment is struggling due to structural and molecular differences between species, and to the lack of knowledge on the underlying mechanisms. Tissue engineering aims to simplify the in vivo systems and reduce the use of animals by recapitulating the physiological conditions of the muscle. To obtain mature fibers, muscle engineering requires tissue-mimicking matrices, high cell density of aligned cells, and proper external stimuli. In this work, we optimize the composition of a previously reported1 photocrosslinkable biomaterial for muscle development. We use bioprinting methods to ensure the fiber alignment and produce large numbers of engineered muscles in a simple, fast and automated way. We obtain tissue-like stiffness structures that recapitulate the native environment and induce muscle fiber differentiation on its own. Compared to 2D, the engineered muscles exhibit upregulated expression of differentiation genes without the conventional chemical stimuli, whose pattern suggests a shift from fast to slow fibers. Those 3D muscles present mature fibers with sarcomere formations and proved functionality. To that end, we activate and modulate the contraction of bioprinted muscles through electrical stimuli, and we present their response to different drugs during mitochondrial respiration tests using Seahorse technology. All in all, we present a scalable and easy-to-differentiate in vitro platform for muscle research with potential applications in atrophy-related diseases.

Keywords

Self-differentiation; Bioprinting; Functional muscle

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Abstract **#389**

3D PRINTED GELMA/HYDROXYAPATITE SCAFFOLDS FOR ENGINEERING BONE-LIKE TISSUE: A POWERFUL TOOL TO PRECLINICALLY VALIDATE NEW PROSTHETIC DEVICES

Rossella Laurano[P][1], Monica Boffito[1], Alice Zoso[1], Roberta Pappalardo[1], Valeria Chiono[1], Gianluca Ciardelli[1]
[1] *Politecnico di Torino - Department of Mechanical and Aerospace Engineering*
Corresponding author's email: rossella.laurano@polito.it

To reduce the need of revision surgeries of bone implants ascribed to loosening phenomena/implant instability, functional coatings are under investigation, requiring standard/reproducible procedures for their preclinical evaluation in a physiological-mimicking environment. In vitro bone tissue models represent a powerful tool for predictive in vitro screening, resulting in a reduction of animal experimentation in agreement with 3Rs principle.
In this perspective, this work was aimed at developing a 3D-printed bone tissue-like through micro-extrusion additive manufacturing of scaffolds from a purposely-engineered bio-ink. Bio-ink formulation was developed by combining gelatin methacryloyl (GelMA, 7%w/V) as polymeric component and inorganic rod-like nano-hydroxyapatite (nHA, 3%w/V) to mimic the bone mineral content. nHA addition did not alter bio-ink thermo-responsiveness as demonstrated by unchanged gelation onset temperature (i.e., 24°C) and kinetics. Differently, differences in the storage modulus before and after irradiation at 365nm (ΔG') showed remarkably higher values for GelMA/nHA compared to GelMA (i.e., ΔG'=5.3kPa vs. 3.7kPa), suggesting the role of nanoparticles as reinforcement filler. By exploiting ink thermo-responsiveness and photo-crosslinking ability, GelMA/nHA formulations were micro-extruded in mild conditions into model square meshed structures with high shape fidelity. Subsequently, printed geometry was refined to morphologically reproduce the cortical/cancellous bone tissues and to maximize cell response. 3D scaffolds (i.e., 25 layers in cylinder-shape constructs with 400 µm pore size) were mechanically characterized showing adequate properties for bone tissue engineering/modelling. Murine fibroblasts (3T3) were preliminarily included in the bio-ink to obtain cellularized constructs. Cell viability tests evidenced the absence of cell damages induced by shear stresses during scaffold fabrication.

References

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Abstract **#391**

BIOACTIVE HYDROGEL COATING TO MODULATE INFLAMMATION AND PROMOTE OSSEOINTEGRATION OF HIP IMPLANTS

Lena Witzdam[P][1], Yannick Meurer[1], Manuela Garay Sarmiento[1], Verena Börger[2], Bernd Giebel[2], Nynke van den Akker[3], Cesar Rodriguez-Emmenegger[1]

[1] DWI – Leibniz Institute for Interactive Materials e.V., RWTH Aachen University, Forckenbeckstraße 50, 52074 Aachen, Germany.

[2] Institute for Transfusion Medicine, University Hospital Essen, University Duisburg-Essen, Virchowstr. 179, 45147 Essen, Germany

[3] Department of Physiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Universiteitssingel 50, 6229 ER Maastricht, Netherlands

Corresponding author's email: witzdam@dwi.rwth-aachen.de

The need of orthopedic implants increased in the last year due to the fact that people becomes older (ageing society). Currently, the long-term stability of orthopedic implants is challenged by two main reasons: generation of microparticles that activate inflammation and bone resorption and bacterial colonization. To overcome these challenges we developed a new coating system for titanium implants that on the one hand prevents denaturation of membrane proteins and bacteria adhesion and on the other hand is able to modulate inflammations and acts as a provisional extracellular matrix for osteoblasts to colonize. The first step is designed by an ultra-thin hydrogel coating based on carboxybetaine methacrylamide (CBMAA) that exhibits excellent antifouling properties and is biocompatible. Furthermore, the hydrogel contains mesenchymal stem cell-derived extracellular vesicles (MSC-EVs), which are released in a self-regulated manner in the presence of matrix metalloproteinases (MMPs) proportional to the inflammation conditions near the hydrogel. Subsequently, the hydrogel is degraded leaving space for the osteoblasts to generate their own extracellular matrix. Bacteria adhesion and biocompatibility tests were performed to check the antifouling properties of the hydrogel. Furthermore, different strategies were explored to link the MSC-EVs to our hydrogel coating. These results demonstrate that the combination of bacteria repellency with modulation of inflammation and promotion of osseointegration is a powerful tool to enhance the long-term application of hip implants.Acknowledgement. This project has received funding from the European Union's Horizon 2020 research and innovation action under grant agreement No. 814495-EVPRO (www.evpro-implant.eu)

Abstract **#392**

REGENERATIVE NANOCLAYS - TRANSLATING A NOVEL BIOMATERIAL WITH RENOVOS BIOLOGICS

Jonathan Dawson[P][1], Agnieszka Janeczek[2]

[1] *University of Southampton*

[2] *Renovos Biologics Ltd*

Corresponding author's email: jid@soton.ac.uk

Clay nanoparticles offer a surprisingly rich array of opportunities for biomaterial design and regenerative medicine that are only beginning to be explored. In aqueous solutions nanoclay particles self-assemble into stiff gels upon contact with physiological ionic solutions. Upon implantation in the body, nanoclay gels provide bioactive environments, through the adsorption of proteins and ions that promote the invasion of cells. Due to their affinity for proteins such as growth factors, nanoclay gels can be applied to deliver, localise, enhance and even pattern activity of growth factors to initiate tissue regeneration. This talk will describe the development and application of nanoclays for growth factor delivery and regenerative medicine including recent work to translate this technology towards a therapy for bone fusion.

Abstract **#393**

DEVELOPMENT OF THAI SILK FIBROIN-BASED HYDROGEL BIOINKS FOR 3D BIOPRINTING APPLICATIONS

Juthamas Ratanavaraporn$_P$[1], Watcharapong Pudkon[1], Chavee Laomeephol[1], Siriporn Damrongsakkul[1,2]

[1] *Biomedical Engineering Program, Faculty of Engineering, Chulalongkorn University*

[2] *Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University*

Corresponding author's email: juthamas.r@chula.ac.th

Silk fibroin, a protein derived from cocoons of Bombyx Mori silkworms, has recently been well-recognized in biomedical applications due to its excellent characteristics such as non-toxicity, non-immunogenicity, biodegradation and mechanical integrity. Our research group have been continuously investigating on the Thai silk fibroin-based scaffolds for tissue engineering and controlled release applications for a decade. Recently, we focused on the application of Thai silk fibroin-based materials as cell carrier and bioink of a 3D bioprinting technique. Various types of Thai silk fibroin-based hydrogel formulations have been developed. Gelation time, viscoelastic properties and biodegradation rate of the Thai silk fibroin-based hydrogels were characterized. Cell encapsulation efficiency and viability of Thai silk fibroin-based hydrogel bioinks were evaluated using mesenchymal stem cells and cell lines. Printability of Thai silk fibroin-based hydrogel bioinks was tested with an extrusion-based 3D bioprinter. The study elucidated the potential of Thai silk fibroin-based hydrogel bioinks for 3D bioprinting of tissue engineered constructs.

Keywords

Thai silk fibroin; bioink; 3D printing

Abstract **#394**

TAILORING GELATIN VIA A CHEMICAL PLATFORM FOR VERSATILITY IN TISSUE ENGINEERING AND BIOFABRICATION

Jasper Van Hoorick[P][1], Agnes Dobos[1], Marica Markovic[2,3], Lana Van Damme[1], Liesbeth Tytgat[1,4], Peter Dubruel[1], Bert Van den Bogerd[5], Aleksandr Ovsianikov[2,3], Sandra Van Vlierberghe[1,4]

[1] Ghent University, Polymer Chemistry and Biomaterials Group, Centre of MAcromolecular Chemistry, Department of Organic and macromolecular chemistry

[2] 3D Printing and Biofabrication Research Group, Institute of Materials Science and Technology, Technische Universität Wien (TU Wien), Vienna, Austria

[3] Austrian Cluster for Tissue Regeneration (http://www.tissue-regeneration.at)

[4] Brussels Photonics, Department of Applied Physics and Photonics, Flanders Make and Vrije Universiteit Brussel, Brussels, Belgium

[5] Antwerp Research Group for Ocular Science (ARGOS), Translational Neurosciences, Faculty of Medicine, University of Antwerp, Wilrijk, Belgium

Corresponding author's email: jasper.vanhoorick@ugent.be

Gelatin is a popular material in the fields of tissue engineering and biofabrication as reflected by the high number of publications covering a plethora of chemical modifications(1). Herein, we report on different chemical modification strategies to tailor gelatin towards specific biofabrication applications. The first photo-crosslinkable gelatin hydrogel, gelatin methacryloyl (Gel-MA), originally developed by our research group was introduced around 20 years ago(2). Ever since, we investigated a number of different modification strategies to further promote the application potential towards various additive manufacturing technologies.

These strategies include further modification of gel-MA via the introduction of additional methacrylates to improve the resolution in multiphoton lithography (gel-MOD-AEMA)(3,4). Besides improving the resolution, also the writing speed and required energy to process a material using multiphoton lithography can significantly be improved by exploiting thiol-norbornene photo- chemistry 5,6 (gel-NB). Moreover, this gel-NB was successfully applied to encapsulate fibroblasts in a multiphoton lithography process with unprecedented cell viability (± 100%)(6). Furthermore, by combining gel-NB with a thiolated gelatin (gel-SH), micro-blood vessels lined with HUVECS could be generated within an adipose tissue-derived stem cell-containing hydrogel with unprecedented precision(7). Besides multiphoton lithography, the gel-NB/gel-SH system also proved promising for conventional deposition-based additive manufacturing for adipose tissue regeneration(8).

In summary, by tailoring the chemical modification of gelatin, it shows unprecedented processing capabilities when exploiting multiphoton lithography and conventional syringe-based biofabrication technologies. Furthermore, the materials could be tailored to serve a number of regenerative applications including adipose tissue(8), placental barrier(4), ocular applications(9), connective tissue(6) and blood vessel formation(7).

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Abstract **#395**

AN ATMOSPHERIC PRESSURE PLASMA JET TO TUNE THE BIOACTIVE PEPTIDE COUPLING TO POLYCAPROLACTONE ELECTROSPUN LAYERS

Alessandro Patelli[1], Alessandro Maffei[2], Annj Zamuner[2], Monica Dettin[2], Paola Brun[3], Paolo Scopece[4]

[1] *Padova University, Dept. Physics and Astronomy*

[2] *Padova University, Dept. Industrial Engineering*

[3] *Padova University, Dept. Molecular Medicine, Unit of Microbiology*

[4] *Nadir srl*

Corresponding author's email: alessandro.patelli@unipd.it

The surface chemistry of scaffolds for tissue regeneration can guide cells growth. In this study [1], we present a novel method to functionalize electrospun Polycaprolactone (PCL) scaffolds allowing the tuning of biomolecule superficial concentration by varying only one process parameter. The method is based on the deposition of NH2 functional groups starting form (3-Aminopropyl) triethoxysilane (APTES) as precursor by a novel Atmospheric Pressure Plasma Jet (APPJ) and by a successive selective covalent linking of these amines with a synthetic Human Vitronectin adhesive cue (HVP). The addition, in the peptide C-terminus, of an aldehyde group ensures the selective ligation by alkylimino-de-oxo-bisubstitution between the primary amine and HVP. By this method, we managed to alter the HVP surface concentration just varying the deposition time of the plasma process; this resulted in different surface coverage of the plasma coating, which in turn led to diverse amount of linked HVP. Coating stability, morphology and coverage was assessed by infrared and photo-electron spectroscopies and by electron microscopy. As a function of the coverage a variation on peptide concentration was revealed by Total Nitrogen method and confirmed by biological assays, which demonstrated an increase of human osteoblasts viability as a function of peptide concentration.

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Abstract **#396**

DECORIN IMPROVES PANCREATIC B-CELL FUNCTION AND REGULATES ECM EXPRESSION IN VITRO

Abiramy Jeyagaran$_P$[1], Aline Zbinden[1], Max Urbanczyk[1], Chuan-en Lu[1], Julia Marzi[1,2], Shannon L. Layland[1], Garry Duffy[3], Katja Schenke-Layland[1,2]

[1] *Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany*

[2] *Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany*

[3] *Discipline of Anatomy and the Regenerative Medicine Institute, School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Ireland*

Corresponding author's email: abiramy.jeyagaran@uni-tuebingen.de

Pancreatic islet transplantation is a promising therapeutic advancement in the treatment of type 1 diabetes; however, a major obstacle remains in supporting cell function post-transplant. To identify possible ECM proteins for the functionalization of islet encapsulation devices, we investigated the presence of ECM in native pancreatic tissues and in our EndoβH3 pseudo-islet system. We identified the small leucine-rich proteoglycan decorin to be strongly co-localized with insulin-producing cells. Supplementation of decorin in the pseudo-islet cultures significantly increased the β-cell glucose response while also downregulating the ECM proteins involved in fibrosis. Furthermore, functionalization of a clinically-approved collagen type 1 (COL1) gel with decorin significantly increased insulin production in response to glucose. Our data proposes decorin as a therapeutic agent to support β-cell function and improve the efficacy of pancreatic islet transplants.

Abstract **#397**

HO-1 OVEREXPRESSED AND HEAT SHOCK-TREATED MSCS IN FROZEN/THAWED CONDITIONS HAVE A NEURAL SPARING EFFECT ON INTRAVENOUS INJECTION IN CANINE ACUTE SPINAL CORD INJURY MODEL

Woo Keyoung Kim[P][1,2], Wan Hee Kim[2], Oh-Kyeong Kweon[2], Byung-Jae Kang[1,2]

[1] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Program for Creative Veterinary Science Research, Seoul National University, Seoul 08826, Korea

[2] Department of Veterinary Surgery, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea

Corresponding author's email: wkkimcoca@snu.ac.kr

The cryopreserved mesenchymal stem cells (MSCs) can be used immediately as a treatment for spinal cord injury (SCI) emergency dogs. However, cryopreserved cells reduce their characteristics such as cellular viability and antioxidant properties. To solve this problem, we potentiated the characteristics of MSCs by heat shock (HS) treatment and further improved antioxidant capacity by heme oxygenase-1 (HO-1) overexpression. As a result of the PCR, these MSCs have increased gene expression related to stemness, anti-inflammatory, and antioxidant. Twelve SCI beagle dogs were randomly assigned into three groups. We injected intravenously HO-1-overexpressed and HS-treated MSCs (MSCs-HO-HS) and compared this group with HS-treated MSCs group, and MSCs group. MSCs-HO-HS group showed significant improvement in hind limb locomotion from one week as compared to other groups. Spinal cords were harvested at four weeks and used for histopathological analyses. MSCs-HO-HS group showed higher expression of neural marker (Nestin, β-III-tubulin) and also revealed less intervened fibrotic changes of the lesion and improved myelination. The results showed that HO-1 overexpressed and HS-treated on MSCs improved the properties of stem cells, further improving the hindlimb motor function and nerve protection seen in the application of existing MSCs. The stem cells are also thought to be useful for early treatment of SCI, given improved motor function from one week. In conclusion, the use of MSCs-HO-HS in SCI first aid is considered to help early neural sparing and further restore hindlimb motor functions.

Keywords

Mesenchymal stem cells; Heat shock; Heme oxygenase-1

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Abstract **#398**

MANIPULATION OF THE MICROSTRUCTURE OF POLY(ETHER)URETHANE : A SIMPLE TOOL FOR CONTROLLING THE ADHESION OF CELLS ON AN ARTIFICIAL POLYMER

Gaëtan Lutzweiler[P1], Nihal Engin Vrana[2], Julien Barthes[3], Pierre Schaaf[3], Michel Rawiso[4]

[1] *University of Rouen*

[2] *Spartha Medical*

[3] *University of Strasbourg*

[4] *Charles Sadron Institute, CNRS*

Corresponding author's email: gaetanlutzweiler@gmail.com

Artificial polymers are widely used in tissue engineering due to their biocompatibility and biodegradation(1). Among them, one can cite poly(ε-caprolactone), PCL, poly(lactic) acid, PLA or polyurethane, PU. Despite their many advantages, the process of cell adhesion on their surface is still challenging and requires often some additional steps during the synthesis in order to incorporate some adhesive ligands or to render the surface more hydrophilic increasing its affinity for cell adhesion(2). All those steps are therefore time consuming and expensive. Herein, we provide a simple way to tailor the adhesion of two cell types namely, NIH 3T3 fibroblasts and Wharton's Jelly-derived mesenchymal stem cells (WJMSCs), by varying the isocyanate-to-polyol ratio during the PU synthesis which is also called the « isocyanate index » (NCOind). We demonstrate that above a given NCOind, all cell types can clearly adhere on the surfaces without any functionalization whereas no adhesion is possible below such threshold. We use both Wide and Small Angle X-ray Scattering (WAXS and SAXS) to correlate this phenomenon to the microstructure of the PU. A phase separation between polyols and isocyanate is shown while the evolution of the nature of the interface between each phase seems to correlate with the adhesive properties of cells(3). Finally, we produce a PU scaffold as a substrate for tissue engineering which allows for both cell adhesion and colonization. To conclude, this approach provides a simple and versatile tool to control cell adhesion onto PU surfaces which could be further used for medical devices or implants.

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Abstract **#399**

NEOCARTILAGE ASSEMBLY WITH ORGANOIDS IN VISCOELASTIC HYDROGELS

João F. Crispim[1], Keita Ito[P][1]

[1] *Eindhoven Technical University*

Corresponding author's email: K.Ito@tue.nl

Current regenerative therapies for cartilage have proved their success in clinics, however they are associated with several drawbacks that require resolution. Optimizing chondrocyte expansion and their in vitro assembly, can potentially reduce the time and costs of these therapies and at the same increase their clinical potential. In this study we developed a suspension expansion protocol for chondrocytes with the addition of notochordal cell matrix (NCM) as supplement. NCM is a matrix rich in collagen type II, GAGs and growth factors. This led to the formation of organoids, similar in composition and organization to cartilage, in large quantities and in a short period of time (12 days). Next, we encapsulated these organoids into alginate hydrogels with different viscoelastic properties to study how viscoelasticity of the matrix affects cartilage formation by the organoids. We observed that in the more viscoelastic hydrogel, the organoids grew and fused together leading to the formation of a homogenous tissue similar to native cartilage in composition and organization in contrast to the more elastic hydrogel formulations. When compared with the standard approach using single cells, encapsulation of organoids led to a higher proliferation, matrix deposition and superior tissue organization. The resulting neocartilage was mechanically superior and stable after 24 days of culture. This study describes a multimodal approach that involves chondrocyte expansion, organoid formation and their in vitro assembly into neocartilage which proved to be superior to the current standard approaches used in cartilage tissue engineering.

Abstract **#400**

SILK-BASED MATERIALS TO CREATE HIGH RESOLUTION THREE-DIMENSIONAL STRUCTURES USING ELECTROHYDRODYNAMIC PRINTING

Martina Viola[P][1,2], Susanna Piluso[2], Marko Mihajlovic[1], Jos Malda[2], Miguel Castilho[2], Tina Vermonden[1]

[1] *Pharmaceutics Department, Utrecht University*

[2] *Department of Orthopaedics, University Medical Center Utrecht (UMCU)*

Corresponding author's email: m.viola@uu.nl

Mimicking the complex hierarchical structure of the extracellular matrix (ECM) has always been a major goal in tissue engineering (TE) approaches [1] [2]. Despite the great advances in biomaterial processing technologies, the main limitation concerns the resolution of the fibers, which hampers the reproduction of ECM.

Here, we combine Silk Fibroin (SF) [5], a highly potent biomaterial that intrinsically has the characteristics of making fibrous structures, with Electrohydrodynamic printing, an innovative 3D printing technique that allows patterning at micro and sub micro scale.

To fabricate these complex structures, Electrohydrodynamic printing applies a voltage between the needle and the collector screen to charge the polymer solution, with a consequent thinning of the fibers, making it possible to reach optimal resolutions for recreating the hierarchical and fibrillar structure of ECM [3] [4].

We have studied SF in its chemical structure to allow a better understanding of the structural and mechanical behaviour of the material before and after printing. We have demonstrated the printability of SF with Electrohydrodynamic printing and, just by tuning the rheological properties, it is possible to obtain straight fibers with a resolution of 10-20 mm. We have also demonstrated that these fibers can be physically crosslinked inducing the formation of b-sheets structure in the protein chain; after crosslinking the fibers are stable and don't dissolve in water.

SF is therefore proving to be an optimal material for this application and is gaining strong interest in soft tissue engineering.

Keywords

Electrohydrodynamic printing ; Silk Fibroin

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Abstract **#401**

NICHE SCAFFOLDS FOR OSTEOCHONDRAL REGENERATION

Sandra Camarero-Espinosa[P][1,2], David Gomes[2], Jip Zonderland[2], Ivo Beeren[2], Marloes Peters[2], Hong Liu[2], Nicole Bouvy[2], Pieter Emans[2], Lorenzo Moroni[2]

[1] *POLYMAT*

[2] *Maastricht University*

Corresponding author's email: sandra.camarero@polymat.eu

Aging worldwide population demands new solutions to permanently restore damaged tissues, thus reducing healthcare costs. Stem cells are a promising alternative due to their differentiation potential into multiple lineages. Yet, better control over cell-material interactions is necessary to maintain tissue engineered constructs in time. In particular, it is crucial to control stem cell quiescence, proliferation and differentiation in 3D scaffolds while maintaining cells viable in situ. Here, we developed a biomimetic scaffold inspired by the mesenchymal stromal cell niche. The multi-compartment scaffold is composed of a hydrogel, aimed at maintaining cell quiescence, encapsulated on a proliferative electrospun cup that is surrounded by a 3D printed scaffold, aimed at differentiation via peptide sequences.

In vitro, cells encapsulated in alginate remained quiescent for a low concentration of RGD peptides bound to the hydrogel matrix. Cells seeded on electrospun meshes were able to proliferate and migrate through the entire mesh thickness, depending on the pore size and fiber diameter of the scaffolds. Cells seeded on the 3D printed scaffolds functionalized with osteogenic and chondrogenic peptides on a gradient manner were able to support cell differentiation, as measured by classical osteogenic and chondrogenic gene markers expression and protein secretion.

In vivo, the scaffolds showed to maintain biocompatibility. The gel compartment served as a pool of quiescent cells that were able to migrate and proliferate on the electrospun mesh. The 3D printed compartment appeared fully infiltrated by neo-tissue and served as a support for cell differentiation with large amounts of collagen and glycosaminoglycan deposition.

Keywords

Stem cell niche; 3D printing; hydrogel

Abstract **#402**

BIOFABRICATION OF VASCULARIZED MYOGENIC SPHEROIDS FOR MUSCLE ENGINEERING

Mendy Minne[P][1], Lise De Moor[2], Lisanne Terrie[1], Laurens Parmentier[3], Sandra Van Vlierberghe[3], Lieven Thorrez[1], Heidi Declercq[1,2]

[1] Tissue Engineering Lab, Department of Development and Regeneration, Faculty of Medicine, KU Leuven, Kortrijk, Belgium.

[2] Tissue Engineering Group, Department of Human Structure and Repair, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium.

[3] Polymer Chemistry and Biomaterials Research Group, Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Faculty of Sciences, Ghent University, Ghent, Belgium.

Corresponding author's email: mendy.minne@kuleuven.be

Engineering muscle tissue is challenging due to the limited degree of diffusion. To overcome this problem, the incorporation of a vascularized network is important. Therefore, a possible strategy is generating vascularized tissue-specific spheroids. The use of these microtissues is advantageous compared to single cells because of their high cell density and accelerated ECM production. When spheroids are combined with a hydrogel, a bioink can be obtained and used for 3D bioprinting. Vascularized spheroids were generated by seeding 7,5x10^5 endothelial cells, fibroblasts and mesenchymal stem cells (MSC) in a 200 µm-microwell system. Myogenic spheroids were generated by seeding 1,0x10^6 myoblasts, whether or not together with MSC and endothelial cells, in a 400 µm-microwell system. After cultivation in expansion medium, spheroids were exposed to differentiation medium. Subsequently, vascularized myogenic spheroids were generated by combining myoblasts, MSC and endothelial cells. To assess the effect of different hydrogel compositions, encapsulation experiments were performed in gelatin-methacryloyl and gelatin-norbornene. Morphology, ECM production and spheroid viability was evaluated.

Stable vascularized and myogenic spheroids could be obtained. Encapsulated spheroids remained viable and histology showed the presence of tissue-specific ECM components. Hydrogel composition had an effect on the morphology. Moreover, different pre-encapsulation stages of the spheroids determined the fiber alignment of the muscle tissue.

During this study it was demonstrated that both stable vascularized and myogenic spheroids can be obtained and encapsulated to use as a part of a printable bioink. Future work will investigate if the bioprinting of vascularized myogenic spheroids leads to functional muscle tissue constructs.

Abstract **#403**

ENGINEERING LARGE AND THICK VASCULARIZED TISSUE CONSTRUCTS

Ying-Cheih Chen[P][1], Yi-Jung Hsu[1], Shih-Yen Wei[1]

[1] *Department of Materials Science and Engineering, National Tsing-Hua University, Hsinchu, Taiwan.*

Corresponding author's email: yisschen@mx.nthu.edu.tw

Nowadays, the main approaches to engineer vascularized tissues are to develop biomaterials combining with cell-based therapy to achieve rapid vasculogenesis, angiogenesis and anastomosis between engineered and host blood vessels. However, the thickness of engineered vascularized tissues in animals were still less than 1 mm, because the hydrogel becomes less permeable with increasing thickness. To overcome this challenge, a diffusion-based computational simulation was used to guide and optimize the geometry of hydrogel structures. Then, human white adipose tissue derived mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs) were encapsulated into gelatin-based prepolymer and patterned into different kinds of three-dimensional (3D) hydrogel structures by photolithography micro-patterning technique. After subcutaneous implantation into mice, the cell-laden hexagonal structures can guide vasculogenesis and accelerated angiogenesis to form uniform distributed vascular networks in the large (diameter ≈ 2cm) and thick hydrogel (> 2 mm) within 7 days in normal and diabetic mice. Moreover, mice islets were integrated into this engineered vascular network, and the process of islet revascularization after transplant were reviewed. After 7 days, transplanted islets were anastomosed and integrated rapidly into the surrounding engineered vasculature, and thereby significantly increased the islet viability and function observed in conventional islet transplantation. In summary, we developed a novel micro-fabrication technique to generate large and thick vascularized tissue constructs and showed its improvement in islets function. This concept could serve as a platform technology for engineering other vascularized tissue or organs.

Abstract **#404**

INDUSTRIALIZATION OF MESENCHYMAL STEM CELL DERIVED EXTRACELLULAR VESICLE MANUFACTURING

Bart Van Dijk$_P$[1]

[1] *Lonza senior scientist*

Corresponding author's email: bart.vandijk@lonza.com

Mesenchymal stem cells (MSCs) have shown promise in regenerative medicine and to treat autoimmune and inflammatory diseases. There is growing evidence that the immunosuppressing function of MSCs can be attributed to MSC-derived extracellular vesicles (MSC-EVs). Additionally, MSC-EVs can be used as a drug delivery system aimed at other diseases. In order for MSC-EVs to be used to treat patients, they must be produced according to good manufacturing practice (GMP), and comply with appropriate regulatory guidelines. Lonza is a partner in the EVPRO consortium, which aims to use MSC-EVs to counteract inflammation of hip revision prostheses. Within EVPRO, two manufacturing platforms are used, either multilayer culture flasks (2D) or hollow fiber bioreactor (3D). In the present study, a detailed gap assessment was performed of both approaches, investigating GMP compliance and scalability of the manufacturing process (i.e., procedures, materials, equipment, preliminary data, process scale, analytical testing).The main gaps identified are removing or substituting starting and raw materials, closing and de-risking the process, establishing an in-process and release testing strategy. Additionally, the 2D process has a scalability gap, as it will likely only be scalable up to phase 2/3 clinical studies. Although there are a considerable amount of gaps in the research processes, if addressed by process development, there are no gaps which could prevent transfer to a GMP facility. Next, Lonza will focus on closing these gaps to industrialize the MSC-EV manufacturing process, and transfer the process into a GMP-like setting. This project was funded by EU Horizon 2020 grant 814495-EVPRO.

Keywords

MSC derived extra cellular vesicles; GMP compliance; Gap analysis

Abstract **#405**

SMALL EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS AND MECHANISMS OF ACTION IN JOINT REPAIR

Wei Seong Toh[P][1,2,3], Shipin Zhang[1,2], Keng Lin Wong[4,2], Sai Kiang Lim[5,6], James Hoi Po Hui[2,3]

[1] *Faculty of Dentistry, National University of Singapore, Singapore*

[2] *Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

[3] *Tissue Engineering Program, Life Sciences Institute, National University of Singapore, Singapore*

[4] *Department of Orthopaedic Surgery, Sengkang General Hospital, Singhealth, Singapore*

[5] *Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore*

[6] *Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

Corresponding author's email: dentohws@nus.edu.sg

The therapeutic efficacy of mesenchymal stromal/stem cells (MSCs) is increasingly attributed to its secretion of trophic factors, particularly 50-200nm small extracellular vesicles (sEVs) which include exosomes. We demonstrated in rat models of osteochondral defects and osteoarthritis that weekly intraarticular injections of MSC-sEVs (100µg) enhanced cell survival, proliferation, and matrix synthesis, while concurrently inhibiting cell apoptosis, inflammation, and matrix degradation to promote osteochondral repair. Using chondrocyte cultures, we attributed some of these cellular activities during EV-mediated joint repair to CD73-mediated adenosine activation of AKT, ERK and AMPK signalling. Additionally, we observed in our rat osteochondral defect model that EV-treated defects displayed a regenerative immune phenotype characterized by higher infiltration of M2 macrophages over M1 macrophages, with a concomitant reduction in pro-inflammatory synovial cytokines IL-1β and TNF-α. In a rabbit osteochondral defect model, we further optimized the number of injections and demonstrated that the combination of MSC-sEVs (200µg) and hyaluronic acid (HA) administered at a clinical practical frequency of 3 intraarticular injections were effective in promoting sustained and functional cartilage repair. Defects treated with MSC-sEVs and HA showed enhanced functional cartilage repair with progressive improvements in gross appearance, histological scores, and mechanical properties. By 12 weeks, defects treated with MSC-sEVs and HA composed mainly hyaline cartilage that are mechanically and structurally superior to HA-treated defects and demonstrated mechanical properties that approximated that of adjacent native cartilage. These findings provide the basis for development of a cell-free MSC-sEV therapy that can be escalated to a clinical protocol for joint repair in patients.

Keywords

Mesenchymal stem cells; Exosomes; Osteoarthritis

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Abstract **#407**

UPSCALING OF A NEXT GENERATION MICROWELL-BASED BETA CELL REPLACEMENT DEVICE

Rick de Vries[P][1], Mojtaba Barzegari[2], Sami Mohammed[1], Adam Stell[1], Carolin Hermanns[1], Marlon Jetten[1], Denise de Bont[1], Omar Paulino da Silva Filho[1], Vijayganapathy Vaithilingam[1], Aurélie Carlier[1], Liesbet Geris[2], Aart van Apeldoorn[1]

[1] MERLN Department of Cell Biology-Inspired Tissue Regeneration, MERLN institute Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

[2] Prometheus, Division of Skeletal Tissue Engineering, Katholieke Universiteit Leuven, Leuven, Belgium

Corresponding author's email: rick.devries@maastrichtuniversity.nl

We are working on an open microwell islet delivery device that aims to maintain pancreatic islet viability upon implantation by stimulating revascularization. Here we report on the standardization, optimization and upscaling of an off-the-shelf beta cell delivery device made from clinically approved polyvinylidene fluoride (PVDF).

Devices showed and even distribution of microwells across the surface, with an average well diameter of ~380 µm, depth of 260 µm, and pore diameters of 50 µm at the bottom and 90x40 µm at the sides. A software application was created to upscale the delivery device towards human-sized implants. Rat islets showed to be both viable and functional after 7 days of in vitro culture in either mouse- or rat-sized implants. Human islets cultured in rat-sized implants showed improved functionality over free-floating control islets after 7 days of culture. Oxygen imaging was used to measure oxygen kinetics in individual islets during normoxia and hypoxia. Local oxygen levels were affected when islets were within 500 µm of one another. Moreover, in silico local oxygen level predictions seem to overlap with in vitro results.

PVDF is a suitable biomaterial for the production of an next version of the microwell islet delivery device. The combination of oxygen imaging and computational modelling offers insight into the optimal islet packing density within the implant, allowing simulation of several future implant designs. Finally, a software application was developed that allows determination of patient-specific implant dimensions and aids physicians to select which size off-the-shelf implant is most suitable for their patient.

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Abstract **#408**

CROSSLINKED HYALURONIC ACID GELS WITH BLOOD-DERIVED PROTEIN COMPONENTS FOR SOFT TISSUE REGENERATION

István Hornyák$_P$[1], Adél Hinsenkamp[1], Zsombor Lacza[2,3]

[1] *Institute of Translational Medicine, Semmelweis University*

[2] *Orthosera GmbH*

[3] *Institute of Sport and Health Sciences, University of Physical Education*

Corresponding author's email: istvan.hornyak@orthosera.com

Hyaluronic acid (HA) is an ideal initial material for preparing hydrogels, which may be used as scaffolds in soft tissue engineering based on their advantageous physical and biological properties. In the present study, two crosslinking agents: divinyl sulfone and butanediol diglycidyl ether were used to investigate their effect on the properties of HA hydrogels. As HA hydrogels alone do not promote cell adhesion on the scaffold, fibrin and serum from platelet-rich fibrin were combined with the scaffold, the aim was to create a material intended to be used as soft tissue implant that facilitates new tissue formation, and degrades over time. The chemical changes were characterized and cell attachment capacity of the protein containing gels was examined using human mesenchymal stem cells, viability was assessed using live-dead staining. FTIR measurements revealed that linking fibrin into the gel was more effective than linking SPRF. The scaffolds were found to be able to support cell adherence onto the hydrogels, and the best result was achieved when HA was crosslinked with divinyl sulfone and contained fibrin. The most promising derivative, 5 % DVS crosslinked fibrin containing hydrogel was injected subcutaneously into C57BL/6 mice for 12 weeks. The scaffold was proven to be biocompatible, remodeling, and vascularization occurred, while shape and integrity were maintained.

Abstract **#410**

TAILORED MULTIMODAL NANOPARTICLES FOR HIGH RESOLUTION STEM CELL TRACKING

Chloe Trayford[P][1], Sabine Van Rijt[1], Darragh Crosbie[1], Vanessa La Pointe[1], Arianne Van Velthoven[1], Mor Dickman[2], Pamela Habibovic[1], Rudy Nuijts[2], Stefano Ferrari[3]

[1] *Maastricht university, MERLN*

[2] *Maastricht university medical center, UMC+*

[3] *Fondazione Banca degli Occhi del Veneto, Venice*

Corresponding author's email: chloe.trayford@maastrichtuniversity.nl

Stem cell (SC)-based therapies hold the potential to revolutionize therapeutics. They enhance the body's natural repair process, but 99.9% are currently suboptimal in safety and efficacy. The dearth of knowledge in the trajectory and state of SCs throughout treatment has prevented both troubleshooting and devising more optimal therapeutic strategies. Nanoparticles (NPs) are ideal to track SC behavior as they can be made multimodal, biocompatible and are easily modified. In our approach, we design NPs tailored for SC monitoring in the corneal regenerative therapy (CLSCT) by exploiting light imaging techniques. Mesoporous silica NPs can be readily multi-functionalized to achieve multimodality, enabling high-resolution positional imaging of SCs. Gold introduced into their cores allows optical imaging, whilst the addition of fluorescent probes into their silica matrices enables fluorescence imaging. Further, by adding fluorescent probes into the pores that are activated by reactive oxygen species (ROS), SC health and potency can be monitored. With such NPs endocytosed into limbal epithelial SCs, the native regeneration mechanism in CLSCT can be observed in real time. This research should not only enable us to more satisfactorily pre-process SCs, rendering this therapy more efficacious, but also influence the optimization of other SC therapies.

Abstract **#411**

3D BIOPRINTING OF FUNCTIONAL PANCREATIC ISLETS FOR BETA CELL REPLACEMENT THERAPY IN TYPE 1 DIABETES

Rick de Vries$_P$[1], Carolin Hermanns[1], Timo Rademakers[1], Vijayganapathy Vaithilingam[1], Sami Mohammed[1], Adam Stell[1], Marlon Jetten[1], Denise de Bont[1], Omar Paulino da Silva Filho[1], Carlos Mota[2], Aart van Apeldoorn[1]

[1] MERLN Institute for Technology-Inspired Regenerative Medicine, Cell Biology-Inspired Tissue Regeneration, Maastricht University, Maastricht, the Netherlands

[2] MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration, Maastricht University, Maastricht, the Netherlands

Corresponding author's email: c.hermanns@maastrichtuniversity.nl

3D bioprinting is an interesting strategy to deliver pancreatic islets towards the human body. To date, only few groups directly 3D bioprinted pancreatic islets inside a hydrogel and assessed cell viability and function.[1-5] However, proper cell functionality was not observed, most likely due to impaired diffusion of insulin. We hypothesize that careful selection of a hydrogel with a relatively open network should lead to the effective diffusion of insulin from hydrogel-encapsulated islets. We studied the diffusion of fluorescently-labeled dextrans (resembling the size of insulin) through different alginate-based hydrogels using fluorescent recovery after photo bleaching (FRAP). In addition, rheology was used to determine the hydrogel mesh size. FRAP indicated that diffusion constants were highest for 1.5% UP alginate, which also showed to hold the largest hydrogel mesh size. Based on these results, INS1E cells (cell line), rat islets or human islets were 3D printed using an Ultimaker 2+ 3D printer modified with a Discov3ry past extruder. Cell functionality was evaluated at both day 1 and day 7, and compared against free-floating controls. All cell types demonstrated to be viable and functional, as the stimulation index exceeded 2. FRAP and hydrogel mesh size measurements are valuable tools to select promising hydrogel formulations for 3D bioprinting. Careful selection of hydrogel characteristics resulted in successful insulin secretion from 3D bioprinted pancreatic islets. We report for the first time on 3D bioprinted pancreatic islets and beta cells which maintain both viability and functionality. Future research will focus on upscaling technique towards a clinically relevant scale.

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Abstract **#412**

GELLAN GUM-HONEY-DIATOM COMPOSITE DELIVERING RESVERATROL FOR CARTILAGE REGENERATION UNDER SEPTIC AND OXIDATIVE STRESS CONDITIONS

Andrea Cochis$_P$[1], Maria A Bonifacio[2,3], Stefania Cometa[3], Piergiorgio Gentile[4], Annachiara Scalzone[4], Alessandro C Scalia[1], Lia Rimondini[1], Elvira De Giglio[2]

[1] *Department of Health Sciences, Center for Translational Research on Autoimmune & Allergic Diseases CAAD, Università del Piemonte Orientale UPO, Novara, Italy*

[2] *Department of Chemistry, University of Bari "Aldo Moro", Italy*

[3] *Jaber Innovation s.r.l., Italy*

[4] *School of Engineering, Newcastle University, UK*

Corresponding author's email: andrea.cochis@med.uniupo.it

Carbohydrate-based porous scaffolds are promising biomaterials supporting cartilage regeneration. In this respect, their composition could be designed to face clinical challenges, i.e., articular load bearing, infections and oxidative stress due to inflammation. Herein, an innovative scaffold has been developed, combining raw materials belonging to different kingdoms of life. Indeed, gellan gum, a bacterial-derived carbohydrate, was blended with a beehive product (Manuka honey) with prominent antibacterial features. Moreover, resveratrol, a phytoalexin with powerful antioxidant activity, was loaded into the silica shells of diatoms, unicellular microalgae with cytocompatible features. We hypothesized that the gellan gum-based composite scaffold holds suitable mechanical properties to support mechanical loading during new cartilage formation, providing anti-infective and antioxidant properties through Manuka-honey supplementation and resveratrol release. The resveratrol-doped scaffold, chemically, morphologically, mechanical and pharmacokinetically characterized, was demonstrated to have mechanical properties in the range of that of natural cartilage (Young's modulus between 130 and 147 kPa), to be cytocompatible and able to promote chondrogenesis by preserving the cells maturation also within oxidative stress conditions by scavenging oxygen and nitrogen active species thus reducing oxidative stress. Resveratrol doping also contributes to enhance the antibacterial protective effect of the Manuka honey towards regenerating chondroblasts even in the presence of Staphylococcus aureus infection due to the ability to preserve the metabolically active cells.

Abstract **#413**

ENHANCEMENT OF MICRORNA-MEDIATED DIRECT REPROGRAMMING OF CARDIAC FIBROBLASTS INTO CARDIOMYOCYTES IN THREE-DIMENSIONAL CARDIAC-LIKE CULTURE MICROENVIRONMENT VERSUS 2D CULTURE

Camilla Paoletti[P][1,2,3], Maria Luna Melis[1,2,3], Carla Divieto[4], Franca Di Meglio[5], Daria Nurzynska[5], Valeria Chiono[1,2,3]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *POLITO Biomedlab, Politecnico di Torino, Turin, Italy*

[3] *Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Turin, Italy*

[4] *Division of Metrology for Quality of Life, Istituto Nazionale di Ricerca Metrologica, Turin, Italy*

[5] *Department of Public Health, University of Naples Federico II, Naples, Italy*

Corresponding author's email: camilla.paoletti@polito.it

Direct reprogramming of fibroblasts into induced cardiomyocytes (iCMs) represents a new promising strategy to restore cardiac functions after myocardial infarction. Recently, our group demonstrated that four microRNAs (miR-1, 133, 208 and 499, termed “miRcombo”), previously studied in mouse model, were effective in direct reprogramming of adult human cardiac fibroblasts (AHCFs) into iCMs [1,2]. Moreover, literature findings suggested that direct reprogramming efficiency increases for cells in three-dimensional (3D) culture substrates [3].

The aim of this work was to analyze the effect of miRcombo transient transfection on AHCFs cultured in 3D fibrin-based hydrogel (5 mg/mL) versus 2D culture plates. After 15 days culture, the expression of Cardiac Troponin T (cTnT), Myosin heavy Chain 6 (Myh6) and Cardiac Troponin I (cTnI) was higher in miRcombo-transfected AHCFs in 3D versus 2D cultures. Interestingly, cardiac markers were slightly upregulated for NegmiR-treated AHCFs in 3D versus 2D cultures, suggesting increased cell plasticity in 3D culture microenvironments.

Additionally, we demonstrated that cardiac markers were upregulated in miRcombo-transfected cells cultured in 2D plates coated with biomimetic natural polymers versus uncoated plates, suggesting that biochemical cues improve direct reprogramming efficiency. Hence, “biomatrix”, a cardiac extracellular matrix produced by AHCFs cultured in vitro [4] was included into 3D-fibrin hydrogels to recreate a cardiac-like microenvironment. Direct reprogramming efficiency of miRcombo-transfected AHCFs cultured in fibrin/biomatrix hydrogels increased as a function of biomatrix concentration (10-100 µg/mL).

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Abstract **#414**

SILENCING TISSUE FACTOR IN MESENCHYMAL STEM CELLS USING PLURONIC MICELLES ENHANCES ITS THERAPEUTIC EFFICACY AND FUNCTION

VigneshKumar Rangasami[P][1,2], Ganesh Nawale[2], Susanna Miettinen[3], Bo Nilsson[4], Yuji Teramura[5,4], Jöns P Hilborn[6], Oommen Varghese[2], Oommen P Oommen[1]

[1] Bioengineering and Nanomedicine group, Faculty of Medicine and Health Technologies, Tampere University, 33720, Tampere, Finland

[2] Translational Chemical Biology Laboratory, Department of Chemistry, Ångström Laboratory, Uppsala University, 751 21, Uppsala, Sweden

[3] Adult Stem Cells Group, Faculty of Medicine and Health Technologies,33014, Tampere University, Finland

[4] Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, SE-75105, Sweden

[5] Department of Bioengineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan.

[6] Polymer Chemistry, Department of Chemistry - Ångström Laboratory, Uppsala University, 751 21, Uppsala, Sweden

Corresponding author's email: vignesh.rangasami@tuni.fi

Stem cell therapies offer promising solutions to treat diseases or conditions for which few treatments exist. There has been interest in harnessing the potential of mesenchymal stem cells (MSCs) however, the clinical trials have been disappointing and inconsistent.[1,2] One of the major reasons for the failure of MSCs in clinical trials is that these cells trigger thrombotic activity upon infusion. The CD142 or tissue factor (TF)[3] expressed on the cell surface is believed to be a master regulator of thrombotic activity and the effect of TF knockdown on these cells were never investigated. In this study, we have engineered a novel pluronic-derived nanoparticle (PSS-NP) for delivering anti-TF siRNA. PSS-TF NPs were able to knockdown (KD) TF with ~75% efficiency without eliciting any toxic effects. The TF-KD MSCs significantly reduced the instant blood mediated inflammatory reaction in human whole blood model as evidenced by reduced platelet aggregation (~3 folds less compared to MSCs) and thrombin anti-thrombin complex formation when compared to the MSCs. Unexpectedly, TF-KD MSCs also enhanced the differentiation efficiency in osteogenic conditions as evidenced by increased mineralization as well as higher expression of genes related to osteogenesis (ALPL, BGLAP, DLX5). The TF-KD MSCs retained their immunosuppressive properties, as evidenced by the suppression of pro-inflammatory cytokines on M1 macrophages. However, the TF-KD MSCs displayed enhanced paracrine signaling when activated by endotoxins. We believe, the results of our study are of great importance and will bring a paradigm shift in performing stem cell-based therapies.

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Abstract **#415**

GUIDING RESPIRATORY EPITHELIUM TOWARDS DIRECTED CILIARY FUNCTION

Jan Weckauf$_P$[1], Anja E. Luengen[1], E. Miriam Buhl[2], Stefan Jockenhoevel[1,3], Anja Lena Thiebes[1]

[1] *Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany*

[2] *Institute of Pathology, Electron Microscopy Facility, RWTH Aachen University Hospital, Aachen, Germany*

[3] *AMIBM, Maastricht University, Geleen, The Netherlands*

Corresponding author's email: weckauf@ame.rwth-aachen.de

Mucociliary clearance (MCC) is an important mechanism of the respiratory system and depends on the globally unidirectional orientation of ciliary beating within the epithelium. For tissue engineered constructs of the respiratory tract it is essential to recreate a correct ciliary beating orientation. However, the unidirectional orientation is missing in current in vitro cultures. In this study, we established an in vitro bioreactor culture system to investigate the influence of mechanical stimuli to respiratory epithelial cells with regard to the global orientation of cilia.

For culture and stimulation, a bioreactor composed of two chambers separated by a PET-membrane was used. The bottom chamber was used for dynamic medium supply, the upper one for the culture and stimulation of human respiratory epithelial cells (HREs). Mechanical stimulation of cells occurred via airflow applied by a syringe pump. Bioreactor cultures with and without stimulation were compared to each other as well as to static controls.

The established bioreactor system enables HRE culture on a PET membrane with dynamic medium exchange and the use of laminar airflow to mechanically stimulate the HREs via defined wall shear stresses. Furthermore, it allows investigation of mucociliary transport using fluorescent microbeads. Immunohistochemistry is used to analyse cell polarization and the orientation of cilia.

Mechanical stimulation of HREs via applied airflow is believed to assist the airway epithelium in correctly orienting the cilia on a global scale. Achieving the unidirectional ciliary beating in vitro represents an important step for the creation of tissue engineered constructs with functioning MCC.

Keywords

Airway tissue engineering; Mechanobiology

Abstract **#417**

UTILISING DYNAMIC ECM-MIMICKING HYDROGELS OF VARIOUS RELAXATION TIMES AND STIFFNESSES FOR KIDNEY ORGANOIDS MATURATION.

Floor Ruiter[P][1,2], Nadia Roumans[1], Thomas Geuens[1], Anika Schumacher[1], Matthew Baker[2], Vanessa LaPointe[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Cell Biology-Inspired Tissue Engineering, Maastricht University, Maastricht, the Netherlands*

[2] *MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, Maastricht, the Netherlands*

Corresponding author's email: f.ruiter@maastrichtuniversity.nl

Induced pluripotent stem cells (iPSCs) differentiated to renal progenitors can self-organise into nephron-like structures when cultured in an air-liquid interface.[1] Although these organoids contain kidney-like structures, their maturity is limited.[2] Moreover, we previously identified an aberrant extracellular matrix (ECM) protein expression (type 1a1 collagen and alpha-SMA) that indicated a possible fibrotic programme. Therefore, making these kidney organoids unsuitable for kidney replacement for patients with chronic kidney disease. Previous results showed a reduction of these aberrant ECM expression when organoids were encapsulated in soft thiol-ene cross-linked alginate hydrogels (180 Pa) for 4 days instead of the air-liquid interface.

To further investigate the hydrogel properties related to this improvement of the renal phenotype, we designed a series of different dynamic covalent hydrogels with various relaxation times or stiffnesses, ranging from approx. 10^1 to 10^5 sec. and/or 100 Pa to 20 kPa respectively. We used cross-linkers that are dynamic under cell culture conditions, resulting in reshuffling of the cross-linker bonds when multiple groups are present.[3] This mimics the dynamic characteristics of the naturally occurring extracellular matrix. The formed kidney organoid were encapsulated in the series of hydrogels and cultured. Their phenotype and the expression of key extracellular matrix proteins was analysed to determine the role of relaxation time and stiffness on organoid culture. Preliminary results shows difference of cell types and ECM expression when encapsulated in a stiffer hydrogel. This study highlights the importance of carefully choosing the hydrogels properties and cellular response on these biomaterials within the regenerative medicine and organoid fields.

Keywords

Dynamic Hydrogels; Organoid Maturation; ECM deposition

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Abstract **#418**

BIOMIMETIC DESIGN OF A CARDIAC TISSUE MODEL: FROM BIOMATERIAL SYNTHESIS TO FUNCTIONAL TESTING

Susanna Sartori$_P$[1], Federico Vozzi[2], Federica Logrand[3], Monica Boffito[1], Mara Brancaccio[3], Gianluca Ciardelli[1]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino*

[2] *Institute of Clinical Physiology, IFC-CNR*

[3] *Department of Molecular Biotechnology and Health Sciences, University of Turin*

Corresponding author's email: susanna.sartori@polito.it

A large number of chemicals remain inadequately tested for their potential cardiotoxic effects. Current approaches rely on large animal models, with limitations in terms of cost and complexity. Support in cardiotoxicity evaluation could be obtained by 3D models effectively mimicking the cardiac tissue. Compared to 2D, 3D cell culture more accurately represents the real cell micro-environment, influencing the spatial organization of cell surface receptors involved in cell-cell and cell-extracellular matrix crosstalk, and providing physical constraints to cells, which affect signal transduction and cell behaviour. 3D cardiac models should replicate also the mechanical properties and chemical composition of the native tissue.

In this work we developed of an innovative poly(ester urethane) (PUR) scaffold mimicking cardiac tissue. The PUR was selected because of its elastomeric-like properties1. The porous scaffold was fabricated by thermally induced phase separation and then functionalized with fibronectin by plasma treatment. The success of fibronectin grafting was assessed via XPS analysis. SEM analysis showed a pore-aligned configuration, with mechanical properties and histoarchitecture resembling the myocardium. Biological characterization with fetal cardiomyocytes highlighted a stable viability for 14 days, including good beating activity and adhesion. The increased levels of phosphorylation of AKT and ERK1/2 ought to account for the prolonged cell survival and beating activity of cardiomyocytes cultured on scaffolds versus TCPs. The modulation of cardiac muscle (prepro-ET-1, MYH7, CTGF) and glucose metabolism (SLC2a1, PFKL) genes indicated a transition phase in cardiomyocyte maturation, with an initial evolution to an adult phenotype and a permanence of some fetal characteristics.

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Abstract **#420**

ENCAPSULATION OF CELLS IN FILAMENT-LIKE HYDROGEL THROUGH VISIBLE LIGHT-INITIATED REACTION AND MICROFLUIDIC SYSTEM

Elham Badali[P][1,2], Mehdi Khanmohammadi[2], Zohre Bagher[3], Maryam Jalessi[2]

[1] *Faculty of Chemistry, Kharazmi University, P. O. Box 15719-14911, 49 Mofateh St., Tehran, Iran*

[2] *Skull Based Research Center and Department, The Five Senses Institute, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran*

[3] *ENT Research Center and Department, The Five Senses Institute, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran*

Corresponding author's email: badaliela@yahoo.com

Hydrogel fibers are structurally and biologically useful devices for the fabrication of filament-like tissues and differentiation of stem cells [1, 2]. We established a cell-laden degradable hydrogel fiber through visible light-initiated crosslinking to differentiate stem cells and fabricate filament-like tissue [2]. The human adipose stem cells (hADSCs)-laden fibers were fabricated by cross-linking phenolic-substituted alginate and gelatin (Alg-Ph and Gela-Ph respectively) in an aqueous solution containing cells through irradiating visible light. The crosslinking of phenolic moieties was mediated by ruthenium(II) tris-bipyridyl dication (Ru(II)bpy and sodium ammonium persulfate (SPS) [2]. The encapsulated hADSCs proliferated and grew within cell-laden hydrogel fiber, and maintained their pluripotency ability the cells formed filament-like constructs. The filament-like tissues covered with an additional heterogeneous cell layer was made by degrading the fiber membrane using alginate-lyase after covering the fiber surface with vascular endothelial cells. Cellular viability preserved more than 90% during Alg-Ph and Gela-Ph hydrogel fiber fabrication and filament-like tissue formation until 18 days. These results demonstrate the feasibility of alginate-based hydrogel fibers obtained through the Ru/SPS-mediated crosslinking system and visible light irradiation for the engineering of filament-like tissues.

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Abstract **#424**

THE BONE POTENTIAL OF HBMSCS IS IN PART ASSOCIATED WITH THEIR IMMUNOMODULATORY CAPACITY

Nathalie Chevallier$_P$[1,2], Laura Coquelin[1,2], Najat Raddi[1,2], Gabriel Windels[1,2], Luciano Vidal[3], Hakim Hocini[1], Hélène Rouard[1,2]

[1] *Univ Paris Est Creteil, INSERM, IMRB, F-94010 Creteil, France*

[2] *EFS, Unité d'Ingénierie et de Thérapie Cellulaire, IMRB, 94017 Creteil, France*

[3] *UMR-1238, INSERM, laboratoire « Sarcomes osseux et remodelage des tissus calcifiés », Université de Nantes, Nantes, France*

Corresponding author's email: nathalie.chevallier@efs.sante.fr

Human bone marrow mesenchymal stromal cells (hBMSCs) associated to biomaterials are currently used in clinic for bone repair. Although the safety of this treatment has been demonstrated in clinical trials, their kinetics of bone formation are heterogeneous (1, 2). Cells from several donors were amplified and grafted either subcutaneously or in-situ into an immunodeficient mouse model and our results indicate that the bone potentials of the cells is donor-dependent. To understand this heterogeneity, we focused on the hBMSCs behaviour and observed that hBMSCs with high bone potential were associated with higher cell survival. To determine their mechanism of action and understand how cell survival is controlled, an RNAseq analysis was performed at 8 hours, 1 and 2 weeks after engraftment. The greatest differences were observed at 8 hours and our results show that cells with reduced bone potential have higher levels of expression of pro-inflammatory cytokines. This is associated with an upregulation of murine neutrophil and NK-cell activation, indicating possible chemotaxis of the host immune system by cytokines secreted by transplanted cells. Differential activation of the host's innate immune system can play a major role in the long-term cell-survival. Neutrophil-depleted mice were used and 24 hours post graft, our results show better cell survival which is associated with an increase in bone formation. In conclusion, our results show that part of the heterogeneity of hBMSCs is due to their ability to regulate the inflammatory response, which has an impact on their survival and therefore their bone potential.

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Abstract **#425**

CHITOSAN/HYALURONIC ACID HYDROGEL VIA HORSERADISH PEROXIDASE REACTION FOR CARTILAGE TISSUE ENGINEERING

Elham Badali$_P$[1,2], Mehdi Khanmohammadi[3], Maryam Jalessi[4]

[1] 1 Faculty of Chemistry, Kharazmi University, P. O. Box 15719-14911, 49 Mofateh St., Tehran, Iran

[2] Kull Based Research Center and Department, The Five Senses Institute, Hazrat Rasoul Akram Hospital

[3] Skull Based Research Center and Department, The Five Senses Institute, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

[4] Skull Based Research Center and Department, The Five Senses Institute, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

Corresponding author's email: badaliela@yahoo.com

Hydrogels derived from natural substrates are emerging constructs for multifunctional microenvironment cell culture and tissue fabrication [1-4]. In this study different compositions of hyaluronic acid (HA) and chitosan (CH) with opposite charges fabricated. In order to increase the interaction between these components phenol moieties conjugated on backbone of HA (HAPH) and CH (CHPH), and the conjugated samples subjected to enzymatic crosslinking via carbodiimide-mediated condensation in the presence of horseradish peroxidase to make hybrid hydrogel scaffolds [1-3]. The physical characteristics of hydrogels including gelation time, enzymatic degradation and water contact angle of hydrogels decreased with increasing HAPH content. The rheological and mechanical properties of the hydrogels showed that moderate concentration of HAPH can have the best results in the hydrogel structure. Hydrogel morphology altered depending on the amount of incorporated HA in CH precursor hydrogel solution and their pore size destitution decreased with increasing HAPH. The cellular studies showed proper cell viability and proliferation for cells on optimum blend hydrogel surface compared with the neat hydrogels. Furthermore, the hybrid hydrogels demonstrated good characteristic for expression of cartilage tissue markers including Col2, SOX9 and ACAN and higher propensity of MSCs to differentiate into cartilage cells comparing to the control samples. Therefore, the results suggest the optimum hybrid hydrogel can provide superior biological microenvironment for chondrocytes in three-dimensional cartilage tissue engineering.

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Abstract **#426**

CHARACTERIZING MANUFACTURED THERAPEUTIC CHONDROCYTES USING PROGNOSTIC BIOACTIVITY MARKERS

Noor Al-Maslamani[P][1], Jude Curran[1], Kai Hoettges[2], George Bou-Gharios[1], James Henstock[1]

[1] *Institute of Aging and Chronic Disease, Liverpool University*

[2] *Department of Electrical Engineering and Electronics, Liverpool University*

Corresponding author's email: N.Al-Maslamani@liverpool.ac.uk

Introduction

Autologous chondrocyte implantation (ACI) is a procedure to treat cartilage defects; chondrocytes are isolated from an unaffected area of cartilage and are then expanded to be re-implanted to repair the defect site. Many aspects of this process are not optimized: in particular, monolayer expansion culture causes chondrocytes to de-differentiate, expressing mesenchymal or stem-like markers as: CD10, CD90, CD105, CD1661, collagen-I, collagen-II and versican. Repair tissue formed from these cells include undesirable fibrous extracellular matrix. We hypothesize the introduction of novel 3D cell expansion techniques (bioreactors) can reduce /eliminate de-differentiation and increase the cells therapeutic potency. To facilitate this investigation, we have developed a prognostic assay using FACS to characterize cell phenotype, enabling a rapid and high-throughput analysis of manufactured chondrocytes.

Methods

A literature review was conducted of the markers associated with chondrocyte isolation, culture, de-differentiation, and trans-differentiation.

Results

FACS markers of healthy chondrocytes, pericellular matrix and mature chondrons (CD 151, collagens II, VI, IX and aggrecan), hypertrophic chondrocytes (collagens I and X). Markers of de-differentiated cells include CD90 (mesenchyme), CD9 (osteochondroprogenitor)2, CD146 (chondroprogenitor)3.

Discussion & Conclusions

We have developed a FACS-based assay to qualify, quantify and characterize the cell types and sub-populations produced during chondrocyte expansion culture. This assay is novel as it identifies the level of de-differentiation under culture conditions and provides a ratio for immature (potentially reparative) chondrocyte/progenitor cells and the mature chondrocytes (surrounded by chondrons). This test allows for a high-throughput screening of cells manufactured under novel and improved bioreactor conditions.

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Abstract **#427**

GENETICALLY ENGINEERED MESENCHYMAL STEM CELLS AMELIORATE CANINE OSTEOARTHRITIS USING PLATELET-DERIVED GROWTH FACTOR AND HEME OXYGENASE-1

Jiwon Oh[P][1], Wan Hee Kim[1], Oh-Kyeong Kweon[1], YeonSung Son[2], Byung-Jae Kang[3,1]

[1] *Department of Veterinary Surgery, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea*

[2] *College of Medicine, Seoul National Univeristy, Seoul 03080, Korea*

[3] *Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Research institute for Veterinary Science, and BK21 PLUS program for Creative Veterinary Science Research, Seoul National University, Seoul 08826, Korea*

Corresponding author's email: jiwon3903@snu.ac.kr

Genetically engineering of mesenchymal stem cells (MSCs) has attracted great interest for osteoarthritis (OA) to enhance cartilage repair. We aimed to investigate the impact of MSCs overexpressing platelet-derived growth factor (PDGF-MSCs) or heme oxygenase-1 (HO-1-MSCs) on OA phenotype in chondrocytes and synovial cells and assess the in vivo efficacy by intraarticular injections in canine OA models. Canine chondrocytes or synovial cells were stimulated with lipopolysaccharide (LPS) to mimic the OA and then cocultured with MSCs, PDGF-MSCs, or HO-1-MSCs. We examined gene expression related to OA pathology; pro-inflammatory, extracellular matrix-degradative/synthetic, or pain-related factors. Furthermore, canine OA models were randomized into four groups: normal saline, MSCs, PDGF-MSCs, and HO-1-MSCs. We then assessed OA symptoms, radiographic OA severity, and serum matrix metallopeptidase (MMP)-13 before and after treatment. Overexpression of PDGF or HO-1 significantly reduced expression of proinflammatory factors, MMP-13, and nerve growth factor elicited by LPS, but increased that of aggrecan and collagen type 2 or tissue inhibitor of metalloproteinases (TIMP) in chondrocytes or synovial cells. Furthermore, co-cultured MSCs have exhibited a high expression of genes to maintain joint homeostasis. In vivo studies showed that OA symptoms were improved after the administration of all MSCs. Also, PDGF-MSCs elicited the most significant improvement of limb function and pain reduction. No remarkable significant difference was found in radiographic assessment and serum MMP-13 change. Targeting genetically modified MSCs focusing on PDGF and HO-1 is an effective strategy to treat OA, which suggests that PDGF-MSCs can be novel bioagents leading to greater improvement of OA symptoms.

Keywords

Mesenchymal stem cell; Osteoarthritis; Platelet derived growth factor

Abstract **#428**

A STRATIFIED TISSUE ENGINEERED CARTILAGE: TOWARDS AN ENHANCED KNEE OSTEOARTHRITIS REPAIR

Georges Kfoury$_P$[1], Marwan Sabban[2], Rami Mhanna[1]

[1] *Biomedical Engineering Program, Faculty of Engineering and Architecture, American University of Beirut, Beirut, Lebanon*

[2] *Department of Anatomy, Cell Biology and Physiological Sciences, Faculty of Medicine, American University of Beirut*

Corresponding author's email: rm136@aub.edu.lb

Articular cartilage disease and injury result in restricted movement, pain and disability in advanced stages. Articular cartilage has a limited ability of self-repair and no known drugs currently exist that fully cure the damaged tissue. Several studies describe the use of tissue engineering tools to construct articular cartilage but most tissue engineered cartilage is made of a mixed cell population without considering the stratified nature of the original tissue. Therefore, we applied tissue engineering techniques to construct stratified cartilage to replace the damaged one. Freshly isolated bovine knee chondrocytes were 3D-cultured in biomimetic alginate sulfate hydrogels which induced their proliferation by 5-fold independent of gel stiffness, while maintaining the cartilage phenotype. Chondrocytes were subjected to different zonal stimuli such as oxygen tension and mechanical stimulations. The mechanical properties of the hydrogel were tested using a texture analyzer (Instron machine) and mechanical stimulations were applied to the whole system using a cell stretching system (STREX). The difference in oxygen tension applied to the system induced different cartilage phenotypes. Low oxygen promoted middle/deep phenotype and high oxygen promoted a superficial zone phenotype. The engineered layers will be bound together to form a single tissue. The stratified biomimetic knee cartilage tissue with phenotypically stable chondrocytes can be developed in vitro and can be used for therapy in case of injury or disease.

Abstract **#429**

DESIGN OF A 3D CORNEAL STROMAL CONSTRUCT USING SUPRAMOLECULAR HYDROGELS FUNCTIONALIZED WITH BIOACTIVE ADDITIVES FOR ENCAPSULATION AND RECRUITMENT OF CORNEAL KERATOCYTES.

Annika Vrehen[P][1,2,3], Jingyi Huang[2,3], Patricia Dankers[1,2,3]

[1] *Department of Biomedical Engineering, Laboratory for Cell and Tissue Engineering, Eindhoven University of Technology*

[2] *Department of Biomedical Engineering, Laboratory of Chemical Biology, Eindhoven University of Technology*

[3] *Institute for Complex Molecular Systems, Eindhoven University of Technology*

Corresponding author's email: a.f.vrehen@tue.nl

The cornea is a transparent avascular tissue which is responsible for refracting and transmitting light into the eye. About 90% of the cornea is composed of the stroma which exists of precisely arranged collagen fibrils. Between these collagen lamellae keratocytes can be found, which are mitotically quiescent cells.[1] In wound repair processes these keratocytes differentiate into a myo-fibroblastic phenotype. Lately, the idea of a bioengineered cornea has gained credibility in the field of ophthalmology. Synthetic hydrogels based on supramolecular moieties allow for material tunability by the incorporation of bioactive additives into the material which encourage the mimicking of the natural extracellular matrix.[2] In this project, we aim to modify bioactive additives with ureido-pyrimidinone (UPy) moieties to design an in-vitro hydrogel-based 3D stromal construct for encapsulation and recruitment of corneal keratocytes.

A collagen type I binding and an integrin binding peptide were synthesized and functionalized with UPy-moieties.[3] Additionally, dendritic structures were synthesized of the collagen binding peptide to mimic multivalent peptide presentation. Various combinations of these peptides were incorporated as bioactive additives within an UPy-based hydrogel. Activated human corneal keratocytes were encapsulated in the hydrogels and cultured for several weeks in 3D. Immunofluorescence revealed the expression of characteristic myo-fibroblastic cell markers: alpha-smooth muscle actin, and vimentin. Moreover, the hydrogels showed proliferated cells and cellular production of various collagen types.

We have successfully designed a 3D-hydrogelconstruct with biological properties that supported the encapsulation of activated keratocytes. Next, this project will be extended with studying the phenotype maintenance of encapsulated primary keratocytes.

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Abstract **#430**

A CAPRINE MODEL OF INTERVERTEBRAL DISC DEGENERATION: A TESTING PLATFORM FOR AN INJECTABLE HYDROGEL

Joseph Snuggs[P1], Christine Rustenburg[2], Kaj Emanuel[2], Simon Partridge[1], Kerstin Mader[1], Chris Sammon[1], Theo Smit[2], Christine Le Maitre[1]

[1] *Sheffield Hallam University*

[2] *VU University Medical Centre, Amsterdam*

Corresponding author's email: j.snuggs@shu.ac.uk

INTRODUCTION: Low back pain affects 80% of the population at some point in their lives with 40% of cases attributed to intervertebral disc (IVD) degeneration. We have recently developed an ex vivo caprine IVD degeneration model that accurately represents the human condition [1]. Here we aim to utilise this model for testing the efficacy of our synthetic, Laponite® cross-linked pNIPAM-co-DMAc, injectable hydrogel (NPgel) [2] to halt or reverse degenerative processes.

METHODS: After establishing the ex vivo model [1], degenerate caprine IVDs were injected with NPgel (+/- mesenchymal stem cells) and subjected to a further 21 days of physiological loading within a bioreactor system. Histology, immunohistochemistry and FTIR imaging were performed subsequently to assess matrix and protein expression changes.

RESULTS: Histological staining identified catabolic matrix and protein expression in degenerated caprine IVDs, similar to changes observed during human IVD degeneration. The injection of NPgel increased the expression of healthy NP matrix proteins, such as aggrecan, and also decreased expression of catabolic factors IL-1β, IL-8, MMP3 and ADAMTS4 compared to degenerate discs that were not injected with NPgel.

DISCUSSION & CONCLUSION: The development of treatments for IVD degeneration is hindered using models which do not closely represent human IVD degeneration. Here we have established a reproducible, large animal model of IVD degeneration. Within which, NPgel halted catabolic processes and initiated regeneration of the IVD, indicating that in the future this may become a viable treatment strategy for IVD degeneration.

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Abstract **#431**

CARTILAGE TISSUE ENGINEERING USING DECELLULARIZED BIOMATRIX HYDROGEL IN MECHANICAL LOADED BIOREACTOR

Mehdi Khanmohammadi[P][1], Sima Bordbar[2], Elham Badali[3], Maryam Jalessi[1]

[1] Skull Based Research Center and Department, The Five Senses Institute, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

[2] Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, 1417743361, Iran

[3] Faculty of Chemistry, Kharazmi University, P. O. Box 15719-14911, 49 Mofateh St., Tehran, Iran

Corresponding author's email: mehdi.khanmohammadi84@gmail.com

Natural extracellular matrix (ECM) has been used as a natural scaffold to improve the repair of numerous tissues (1-3). Decellular ECMs can induce and promote cellular fate such as proliferation and differentiation of the stem cells. The decellular matrix gelled by neutralization in the physiological conditions which prove the injectability of the produced scaffold. The transforming growth factor-beta (TGF-β) superfamily is a key mediator of MSC chondrogenesis. Here, we investigated the decellularized cartilage hydrogel containing alginate microsphere Loaded by TGF-β to the improvement of chondrogenic differentiation of human MSCs. The TGF-β encapsulated in alginate microspheres showed release times extending up to 6 days. Decellularized hydrogel seeded with MSCs and TGF-β containing microspheres developed comparable chondrogenic gene expression to constructs supplemented with TGF-β continuously in culture media or with hydrogel without any TGF-β, whereas constructs with TGF-β directly encapsulated in the ECM without microspheres had inferior properties. Histological studies also show the appropriate chondrogenic differentiation for the hMSCs in the test group. Also when these constructs implanted in defects that were prepared from the cow femur knee, constructs containing TGF-β microspheres resulted in superior cartilage matrix formation to groups without or with TGF-β added directly to the gel. This study demonstrates that the controlled local delivery of TGF-β is essential to neocartilage formation by MSCs and that further optimization is needed to avert the differentiation of chondrogenically induced MSCs towards a hypertrophic phenotype.

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Abstract **#433**

NANOPARTICLES BIOCOMPATIBILITY ON INNER EAR CELLS DERIVING FROM THE ORGAN OF CORTI

Laura Astolfi[P1,2], Mariarita Candito[1,2], Erica Gentilin[1,2], Edi Simoni[1,2], Serena Danti[3,4,2]

[1] *Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua*

[2] *INSTM, National Interuniversity Consortium of Materials Science and Technology, Via G. Giusti 9, 50121 Florence, Italy*

[3] *Dept. of Civil and Industrial Engineering, University of Pisa, Italy*

[4] *OtoLab, Otorhinolaryngology, Audiology & Phoniatrics Unit, Azienda Ospedaliero-Universitaria Pisana (AOUP), Pisa, Italy*

Corresponding author's email: laura.astolfi@unipd.it

Nanotechnologies applied to the inner ear involve different applications, among which the development of drug delivery systems and the production of new materials useful to improve the efficiency of innovative cochlear implants. The purpose of this study was to examine the biocompatibility of different nanoparticles, lithium niobate, barium titanate and chitin nanofibers (nanochitin) on a cell line derived from an Immortomouse ™ organ of Corti, the OC-k3. These cells mimic the sensorial inner ear tissues because they express markers of hair and supporting cells and not those typical of neuronal cells. Cell viability, cell morphology, apoptosis and oxidative stress were tested on OC-k3 cells treated with nanoparticles up to 48 hours. The results showed that lithium niobate and nanochitin did not affect the viability of OC-k3 cells up to 48 hours. Further tests showed that all nanoparticles did not affect cell morphology, apoptosis and oxidative stress. Lithium niobate, barium titanate and nanochitin show a good biocompatibility on sensory cells, therefore they could be used to develop an innovative cochlear implant electrode based on a new piezoelectric nanomaterial obtained by combining these nanoparticles. This study was performed as part of the Italian Health Ministry project “New self-powered devices for cochlear stimulation based on piezoelectric nanomaterials” (RF-2011-02350464), the “4NanoEARDRM” project funded under the frame of EuroNanoMed III, and by the Italian Ministry of Education, University and Research (MIUR), Italy [grant n. PRIN-2010S58B38_004].

Abstract **#434**

OPTICALLY CLEAR, MECHANICALLY STABLE CONSTRUCTS FOR CORNEAL TRANSPLANT – A HYBRID APPROACH

Rhiannon Grant[P][1], Antonio Feliciano[1], Gozde Sahin[1], Matthew Baker[1], Stefan Giselbrecht[1]

[1] *Maastricht University*

Corresponding author's email: r.grant@maastrichtuniversity.nl

Globally, donor tissue falls far short of meeting demand for corneal transplants; currently less than 2% of patients in need receive a donor cornea. Synthetic alternatives to donor tissue are on the market, but these are a last resort measure due to their high risk of rejection. Additionally, these synthetic corneas do not allow the cells of the eye to repopulate the region. As a result, tissue engineers are focusing on creating materials which support corneal cell expansion and function. These materials are often very successful in this aspect, however fail to take into account factors such as optical transparency, refractive indices and the necessary mechanical characteristics to support surgical considerations such as suturing and intraocular pressure.

Here, we present a hybrid construct which takes these considerations into account. An optically clear hydrogel was combined with supportive patterned electrospun matrices to provide a construct capable of supporting both corneal cell expansion and function as well as surgical suturing and necessary pressure prerequisites. Several material combinations were tested with human corneal keratocytes and results validated using immunohistochemistry and qRT-PCR. Additionally, a novel mechanical characterization was performed to assess suturability of the construct.

Results indicate that these constructs hold promise for corneal transplant, providing an alternative to the shortcomings of synthetic corneas and the global shortage of donor tissue.

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Abstract **#435**

YAP DRIVES NANOPARTICLE-CELL ASSOCIATION: TOWARDS THE UNDERSTANDING OF BIO-NANO INTERACTIONS THROUGH MECHANOBIOLOGY

Marco Cassani[P][1], Soraia Fernandes[1], Jorge Oliver-De La Cruz[1], František Foret[2,3], Francesca Cavalieri[4,5], Frank Caruso[6], Giancarlo Forte[1]

[1] *International Clinical Research Center, St Anne's University Hospital, Brno, Czech Republic*

[2] *Department of Bioanalytical Instrumentation, CEITEC Masaryk University, Brno, Czech Republic*

[3] *Department of Bioanalytical Instrumentation, Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*

[4] *School of Science, RMIT University, Melbourne Victoria, Australia*

[5] *Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata", Via Della Ricerca Scientifica, Rome, Italy*

[6] *ARC Centre of Excellence in Convergent BioNano Science and Technology, Department of Chemical Engineering, The University of Melbourne, Parkville, Victoria, Australia*

Corresponding author's email: marco.cassani@fnusa.cz

Understanding the complex interactions between cells and nanoparticles has been one of the main objectives of nanomedicine since its dawn (1). The physico-chemical properties of nanoparticles have been studied over the past decades for improving the delivery of therapeutic anti-cancer drugs (2-4). Although significant, these efforts have produced poor clinical translation of nanoparticle-based therapies (5).

Therefore, a shift toward new paradigms, in which the inner molecular processes of the cells take the lead in the interpretation of bio-nano interactions, is desirable. In this context, the study of cell mechanics has emerged as a promising area of investigation (6). Indeed, the mechanical properties of cells have been recently proposed as new prognostic factors in cancer growth and dissemination (7). Mechanotargeting and mechano-therapeutics made their way through medical vocabulary as a new class of drugs and treatments targeting mechanically activated pathways involved in pathologies (8-9). By using nanoparticles with tunable size and surface coating, we demonstrate that the inhibition of Yes-associated protein (YAP) – a key protein of mechano-regulated Hippo pathway responsible for cell stiffness regulation (10) - affects nanoparticles uptake in cancer cells. Remarkably, nanoparticles internalization is independent of YAP inhibition in healthy cells, where its activity is less pronounced. In conclusion, our study represents a proof-of-concept that the internalization of nanoparticles in target cancer cells might be controlled by tuning cell mechanosensing pathways, ultimately improving the specificity of the nanotherapy.

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Abstract **#437**

REGENERATIVE PROPERTIES OF TOPICALLY APPLIED 3D ELECTROSPUN NANOSCALED BIOACTIVE GLASS FIBERS ON DIABETIC ORAL MUCOSAL DEFECTS

Noha Eltantawy Ahmed Baiomy Elshazly$_P$[1], Manal Saad[2,3], Abdelaziz Khalil[4], Marco Patruno[5], Jui Chakraborty[6], Rania ElBackly[7], Ayat Hamdy[7], Mona Marei[7]

[1] *Tissue Engineering Laboratories, Faculty of Dentistry, Alexandria University, Egypt*

[2] *Tissue engineering laboratories,Faculty of Dentistry, Alexandria University, Egypt.*

[3] *Oral Biology, Faculty of Oral and Dental Medicine, Ahram Canadian University, Giza , Egypt*

[4] *Oral and Maxillofacial Surgery, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.*

[5] *Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (PD), Italy.*

[6] *Bioceramics and Coating Division, Central Glass and Ceramics Research Institutes, Kolkata India.*

[7] *Tissue Engineering laboratories Faculty of Dentistry, Alexandria University, Alexandria, Egypt.*

Corresponding author's email: noha.shazly@dent.alex.edu.eg

Diabetes triggers chronic inflammation, declines the antibacterial action and angiogenesis leading to protracted wound healing. Bioactive glass manifested high regenerative efficacy when used in bone regeneration (1). This study aimed to assess the regenerative efficacy of novel bioactive glass nanofibers (BGnf) on surgically created alveolar mucosal wounds of the diabetic rabbit model. Glass nanofibers(500-900nm):(1–2mol% of B2O3, 68–69 mol% of SiO2, and 29–30 mol% of CaO) were synthesized via the sol-gel technique followed by electrospinning of the glass/polymer sol and heat-treatment of fibers at 700C(2). Fibers analysis broadcasted its amorphous, cross-linked structure and bioactivity. Following that, 12 healthy New Zealand rabbits were successfully subjected to chemical induction of type I diabetes. Two weeks after diabetes confirmation, two groups of bilateral elliptical maxillary mucosal defects (10 * 3.5 mm) were created. The defects of the experimental group were grafted with BGnf(n=12), while the other group was the control(n=12). To evaluate fibers regenerative efficacy, three different assessments (clinical, histological, and immune-histochemical) were performed at 1, 2 and 3 weeks' time interval(2). After 1 week, BGnf treated wounds showed complete wound regeneration with significantly high-level VEGF (14.08±3.88%) and collagen I% (6.12±0.49%) expression. Contrariwise, the control group showed suppurative exudates with inflammatory cell infiltration at lamina propria and lower VEGF(3.92±0.222%) and collagen I(3.88±1.934%) expression. After 3 weeks, VEGF and collagen I of BGnf group also recorded a higher expression than the control group. In conclusion, BGnf stimulates in- situ soft tissue regeneration, neovascularization and antibacterial action in the wet oral environment in diabetic patients.

Keywords

Bioactive glass nanofibers; Diabetes mellitus; Oral mucosa

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Abstract **#438**

CHITOSAN-POLYVINYL ALCOHOL AND SILK FIBER SEEDED WITH DIFFERENTIATED KERATINOCYTE FOR SKIN TISSUE REGENERATION

Mehdi Khanmohammadi[P][1], Arash Goodarzi[2], Elham Badali[3]

[1] Skull Based Research Center and Department, The Five Senses Institute, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

[2] Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fars, Iran

[3] Faculty of Chemistry, Kharazmi University, P. O. Box 15719-14911, 49 Mofateh St., Tehran, Iran

Corresponding author's email: mehdi.khanmohammadi84@gmail.com

Fiber mat containing cell interactive molecules offers the ability to deliver the cells and bioactive molecules in wound bed, which will help to achieve a high therapeutic treatment (1-3). In this study, co-electrospun hybrid of polyvinyl alcohol (PVA), chitosan (Ch) and silk fibrous mat was developed and their wound healing potential by localizing bone marrow mesenchymal stem cells (MSCs)-derived keratinocytes on it was evaluated in vitro and in vivo. The fabricated fibrous Ch-PVA + Silk hydrogels were characterized for their structural, mechanical and biochemical properties. The shape uniformity and pore size of fibers showed smooth and homogenous structures of them. Fourier transform infrared spectroscopy (FTIR) verified all typical absorption characteristics of Ch-PVA + Silk polymers as well as Ch-PVA or pure PVA substrates. The contact angle and wettability measurement of fibers showed that mats found moderate hydrophilicity by addition of Ch and silk substrates compared with PVA alone. The mechanical features of Ch-PVA + Silk fibrous mats increase significantly through co-electrospun process as well as hybridization of these synthetic and natural polymers. Higher degrees of cellular attachment and proliferation obtained on Ch-PVA + Silk fibers compared with PVA and Ch-PVA fibers. In terms of capability of Ch-PVA + Silk fibers and MSC-derived keratinocytes, histological analysis and skin regeneration results showed this novel fibrous construct could be suggested as a skin substitute in repair of injured skin and regenerative medicine applications.

Keywords

Hybrid chitosan-PVA-silk fiber; MSC-derived keratinocytes;; Skin tissue regeneration.

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Abstract **#439**

TISSUE ENGINEERING OF THE TEMPOROMANDIBULAR JOINT CONDYLE: AN ANATOMICAL HISTOLOGICAL INVESTIGATION. THE DEPARTMENT OF MATERIALS, SCHOOL OF NATURAL SCIENCES, THE UNIVERSITY OF MANCHESTER, UK. A. FREED, M.O'BRIEN, J. GOUGH AND S. CARTMELL.

Amara Freed$_P$[1]

[1] *Student*

Corresponding author's email: amara.freed@postgrad.manchester.ac.uk

The temporomandibular joint (TMJ) is a complex joint which is made up of the articular disc and condyle (1). Temporomandibular disorders (such as clicking, locking, dislocation, and degeneration) affect 10.5 million people worldwide (2). The current gold standard for repair is surgery which carries risks, lengthy downtime and long-term pre/post assessments. Tissue engineering is a promising alternative for condylar repair and replacement. In order to regenerate the condyle, the anatomical structure needs to be understood. As current literature is limited in this area, this study investigates the anatomy of four rat condyles using histology.

Two rats were dissected (3). Haematoxylin and eosin (H&E), collagen type II, picrosirius red (PSR) and alcian blue (AB) stains were applied. The H&E stain identified the following four zones in the condyle; fibrous layer, proliferative layer, chondrocytic layer and the hypertrophic layer.

The PSR stain highlighted collagen in the condyle but showed no collagen type I or III. Collagen type II marker stain showed type II collagen present throughout the four layers of the rat condyle. The AB stain revealed sulphated acid mucopolysaccharides at 0.2M throughout all four layers. 0.5M highlighted strongly sulphated acid mucopolysaccharides present throughout all four layers. Keratan sulphate was strongly present in the fibrous layer at 0.9M.

The PSR stain showed no traces of collagen type I and scarce type III which challenges previous literature (1). The AB stain showed a variance of proteins throughout the condyle. These differences between human and rat tissue are currently being investigated further.

Keywords

mandibular condyle; histopathological stains; temporomandibular joint

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Abstract **#440**

POLY (GLYCEROL SEBACATE): AN ELASTOMER FOR REGENERATION OF PALATAL DEFECTS IN RABBITS

Rania El Backly[1,2], Ayat Hamdy$_P$[2], Ahmed El Mahalawy[3], Adham El Ashwah[3], Samir Nouh[4,2], Manal Saad[2,5], Samer Zaky[6]

[1] *Endodontics, Conservative Dentistry, Faculty of Dentistry, Alexandria University, Alexandria, Egypt*

[2] *Tissue Engineering laboratories, Faculty of Dentistry, Alexandria University, Alexandria, Egypt*

[3] *Oral and Maxillofacial Surgery, Faculty of Dentistry, Alexandria University, Alexandria, Egypt*

[4] *Veterinary Surgery, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt*

[5] *Oral Biology, Faculty of Oral and Dental Medicine, Ahram Canadian University, Giza, Egypt*

[6] *Restorative Dentistry and Comprehensive Care Center for Craniofacial Regeneration (CCR), Pittsburgh University, Pennsylvania, USA*

Corresponding author's email: rania.elbackly@alexu.edu.eg

The aim of this study was to evaluate the bone regenerative capacity of a soft, load-transducing elastomeric scaffold; Poly(glycerol sebacate)(PGS) in surgically created palatal defects simulating a cleft palate condition in New Zealand white rabbits. A total of 18 rabbits were used. A palatal defect model was created posterior to the maxillary second incisors (n=8). After optimization, the final 5x2.5x4mm3 defect reaching to -but not perforating- the nasal mucosa was created in 11 rabbits; sham control (n=5) or PGS (n=6). Samples were retrieved after 4 and 8 weeks for CBCT, histological and histomorphometric analyses. Results showed that dimensions of the defect grafted with PGS showed significant reduction after 8 weeks, but sham defects showed a decrease in coronoapical dimensions but increase in mediolateral dimensions. Bone density (2096.4HU±83.62) and mean bone fill% (70.46±1.73) were significantly higher for PGS than sham defects (1807.51HU±25.10) and (57.55±4.90) at 8 weeks, respectively. These values showed increase for PGS from 4 to 8 weeks while they decreased for the sham group indicating advanced bone resorption. Histologically, PGS defects showed almost closure and preservation of the external contour. PGS scaffolds showed a clear enhancement of recruitment of osteoblasts and osteoclasts, significantly increased angiogenesis as well as enhanced osteoid matrix deposition. This study introduced a novel application for PGS in the maxillofacial region. Mimicking the osteoid matrix, it allowed the stimulation of recruited cells into an osteogenic lineage and dynamic bone remodeling while preserving contour and mid palatal suture anatomy which are crucial for cleft palate management.

Keywords

Rabbit palatal defects; Bone regeneration; poly (glycerol sebacate) elastomeric scaffold

Abstract **#443**

FABRICATION OF COMPLEX 3D CELL CULTURE SUBSTRATES FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE APPLICATIONS

Gozde Sahin[P][1], Nello Formisano[1], Rhiannon Grant[1], Roman Truckenmüller[1], Stefan Giselbrecht[1]

[1] *Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands*

Corresponding author's email: g.sahin@maastrichtuniversity.nl

Numerous studies clearly indicate the existence of a strong influence of physical and chemical cues in native extracellular matrix (ECM) on cell behavior. In particular, micro- to nanoscale topography features in the natural ECM can significantly affect cell morphology, adhesion, migration, proliferation and differentiation [1]. In order to study such spatially defined cell-matrix interactions and to help the formation of biologically functional tissue analogues in vitro [2], there has been an increasing demand for advanced cell culture platforms able to emulate native cellular environments.

A variety of techniques can be used to create micro/nanotopographies on planar substrates. Many studies have confirmed the advantages of such biomimetic substrates as compared to conventional flat cell culture-ware [3]. However, the integration of topographies onto non-planar, curved substrates, which reflects in vivo-like tissue geometry more closely [4], has not been fully investigated due to fabrication limitations.

To address these challenges, we developed an advanced fabrication process that allows us to create complex 3D substrates with both curvature and cell-scale or even subcellular features. Our process is based on an initial formation of micro- or nanotopographies on flat polymer films by conventional nanoimprint lithography and successive curvature formation by a solid-state forming process called 'microthermoforming' [3]. This allows to transform pre-structured planar thermoplastic polymer films into curved substrates which have radii milli- or micrometer range, or at a smaller scale. The developed substrates are expected to better mimic complex in vivo architectures and to provide engineered cell culture substrates for various tissue engineering applications.

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Abstract **#442**

ORGAN CULTURE AND BIOREACTORS FOR ASSESSING BIOMATERIALS FOR TISSUE REGENERATION

Mauro Alini[P][1]

[1] *AO Research Institute*

Corresponding author's email: mauro.alini@aofoundation.org

Advanced biomaterials and tissue engineered constructs have been developed to improve tissue repair; nevertheless, their clinical translation has been hampered, also by the lack of reliable in vitro models suitable for pre-clinical screening of new implants and compounds mimicking the in vivo situation.

Tissue regeneration is strongly influence by the mechanical properties and behavior of biomaterials, which can be completely different when tested in "isolation" or in a biological context. Therefore, it is important to evaluate the performance of such advance biomaterials in in vitro models, which reproduce closely the in vivo tissue status.

To such end, we have developed several complex organ models (cartilage, intervertebral disc, bone, etc.) which include, not only the tissue part, but the tissue is cultured within a bioreactor, reproducing loading patterns similar to the in vivo microenvironment. Here, we will focus on bioreactor systems that transmit a mechanical stimulus, as this is a key parameter in the homeostasis of various musculoskeletal tissues, such as bone, cartilage, tendon, and intervertebral disc.

By testing regenerative therapies under conditions that are closer to the ones encountered in vivo, bioreactors can provide a useful screening tool and standardization opportunities for the evaluation of various biomaterials, but as well as cell types, drugs, or tissue engineered products. This will allow to reduce the number of samples for the final in vivo evaluation, allowing the 3R philosophy approach to be implemented.

Abstract **#447**

NON-DESTRUCTIVE MOLECULAR IMAGING APPROACHES IN CARDIOVASCULAR TISSUE-ENGINEERING

Julia Marzi[1,2,3], Eva Brauchle[1], Hannah Bauer[1], Anna Biermann[4], Aurelie Serrero[5], Sylvia Dekker[6,7], Carlijn Bouten[6,7], Martijn Cox[5], Anthal Smits[6,7], Ulrich Stock[4,8,9], Katja Schenke-Layland[1,2,3]

[1] *Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany*

[2] *Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University Tübingen, Germany*

[3] *NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*

[4] *Department for Thoracic and Cardiovascular Surgery, Johann Wolfgang Goethe University, 60596 Frankfurt am Main, Germany*

[5] *Xeltis BV, 5612AR, Eindhoven, The Netherlands*

[6] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands*

[7] *Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven, Netherlands*

[8] *Department of Cardiothoracic Surgery, Royal Brompton and Harefield Foundation Trust, Harefield UB96JH, United Kingdom*

[9] *Imperial College London, London SW72AZ, United Kingdom*

Corresponding author's email: julia.marzi@uni-tuebingen.de

Durable and non-immunogenic tissue-engineered heart valve replacements are not yet commercially available. Thus, a better understanding of developmental and regulating mechanisms of cardiovascular tissues is essential to develop new implant materials. Moreover, cardiovascular tissue transplants or tissue-engineered grafts need to be monitored before transplantation.

Towards this aim, Raman microspectroscopy and multiphoton imaging were established as marker-independent, non-destructive techniques for quality assessment of cardiovascular transplants. Allogenic and xenogenic cryopreserved heart valves as well as different tissue-engineered polymer scaffold-based heart valve grafts were implanted in sheep models and explants of different timepoints were analyzed by single-point Raman spectroscopy, Raman imaging as well as multiphoton imaging. Transplanted cryopreserved heart valves, were analyzed in regards to tissue integrity and immunogenicity. Moreover, quantitative monitoring of interstitial cryoprotectant concentrations was performed for quality control. In scaffold-based TE heart valves, Raman imaging was applied to investigate structural changes over implantation time and in different leaflet regions. In addition to marker-independent localization of scaffold and tissue structures such as collagen fibers, nuclei or fibrin, this technique allows for a detailed molecular analysis based on specific Raman signatures. Thus, implants could be analyzed in regards to biomaterial degradation and collagen maturation on a molecular level.

Raman analysis combined with multivariate data analysis tools allowed for the determination and characterization of structural extracellular matrix changes, tissue infiltration and formation, scaffold degradation and real-time quantification of residual cryoprotectants.

This work affirms the potential of Raman techniques for future applications in in situ quality assessment in cardiovascular implants.

Abstract **#449**

COLLAGEN ELECTROSPINNING FOR OSTEOPOROTIC FRACTURES

Clarissa Tomasina$_P$[1], Giorgia Montalbano[2], Priscila Melo[2], Sonia Fiorilli[2], António Azevedo[3], Paulo Quadros[3], Chiara Vitale Bovarone[2], Sandra Camarero-Espinosa[1], Lorenzo Moroni[1]

[1] *MERLN Institute for for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands*

[2] *Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy.*

[3] *Fluidinova S.A, Rua Eng. Frederico Ulrich, Moreira da Maia, Portugal*

Corresponding author's email: c.tomasina@maastrichtuniversity.nl

Osteoporosis is a degenerative disease caused by an imbalance between bone formation and resorption, leading to bone weakening and fractures. One of the affected areas is the pelvis whose fractures are not treated since they require extensive interventions and long recovery. Here, we fabricated an injectable electrospun scaffold made of type I collagen incorporating mesoporous bioactive glasses (MBG) and nano-hydroxyapatite (nano-HA) to promote bone regeneration. Collagen was extracted from rat tails and scaffolds were fabricated using electrospinning. First, different solvents were evaluated and the collagen quality was investigated after fabrication via SDS-page and circular dichroisms. Then, MBG and nano-HA were incorporated from 5wt% to 15wt% and the mats were evaluated in terms of mechanical properties and cytocompatibility. Last, scaffolds were produced using direct writing electrospinning (DWESP), focusing the jet to a point to create 3D scaffolds. Grid and concentric pattern were fabricated, searching for the design that could hold the pressure when injected to the fracture. The ideal solvent resulted to be acetic acid:water, whereas PBS20x:ethanol and acetic acid:ethyl acetate:water did not allow the complete collagen dissolution and therefore were not processable. The resulting scaffolds had a fiber diameter of ~200 nm and the presence of collagen bands was confirmed by SDS-page. MBG and nano-HA were incorporated after sonication up to 15wt%. The two DWESP designs were optimized by varying diameters and dimensions testing also their injectability. In conclusion, collagen could be dissolved and spun successfully through electrospinning and DWESP with or without nanoparticles to be injected into osteoporotic fractures.

Abstract **#451**

ELECTROSPUN FIBRES WITH HYALURONIC ACID-CHITOSAN NANOPARTICLES PRODUCED BY A PORTABLE DEVICE

Carla V. Fuenteslópezp[1], Hua Ye[1]

[1] *Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, OX3 7DQ, UK*

Corresponding author's email: carla.fuenteslopez@eng.ox.ac.uk

Electrospinning is a versatile technique to produce nano and micro scale fibrous scaffolds for tissue engineering and drug delivery applications. This research aims to demonstrate that hyaluronic acid-chitosan (HA-CS) nanoparticles can be electrospun together with a mixture of polycaprolactone (PCL) and gelatine (Ge) using a portable device to create scaffolds for tissue repair. A range of polymer solutions of PCL and Ge at different weight/volume concentrations and ratios were electrospun and characterised. Fibre-cell interaction (F11 cell line) was evaluated based on cell viability and proliferation. Three polymers (8% w/v at 85:15 and 70:30 PCL:Ge ratio, and 10% w/v 50:50 PCL:Ge) were selected and electrospun into different fibre arrangements: random or aligned. HA-CS nanoparticles were synthesised and characterised, and used to functionalise the selected electrospun fibres (8%, 70:30). The optimal dosage (1x10^-2 mg/ml) of NPs was determined using a cytotoxicity assay, prior to embedding these into the fibres as either surface modification or blend. Fibres with blended NPs delivered a higher cell viability than unmodified fibres, while those NP-coated fibres resulted in a higher cell proliferation (72h) than the NP-blended ones. These biocompatible scaffolds allow cell attachment, maintain fibre arrangement, promote directional growth, and yield a higher cell viability.

Abstract **#452**

DECELLULARIZED EXTRACELLULAR MATRIX FROM HUMAN BONE AS VERSATILE PLATFORM TO STUDY THE COMPLEXITY OF MSC – MATRIX INTERACTIONS IN VITRO

Rita Pereira$_P$[1,2], Drenka Trivanović[1,2], Dominic Gehweiler[3], Maximilian Rudert[4], Marietta Herrmann[1,2]

[1] *IZKF Research Group Tissue Regeneration in Musculoskeletal Diseases, University Clinics Wuerzburg, Germany*

[2] *Bernhard-Heine-Center for Locomotion Research, University of Wuerzburg, Germany*

[3] *AO Research Institute Davos, Switzerland*

[4] *Department of Orthopaedic Surgery, Koenig-Ludwig-Haus, University of Wuerzburg, Germany*

Corresponding author's email: r-pereira.klh@uni-wuerzburg.de

Despite the considerable volume of research into the biology and regenerative potential of MSC, the lack of suitable models that recapitulate the in vivo situation has led to poor clinical translation1. Decellularized constructs have the ability to present tissue-specific ECM components in their native organization.

In this work, three different constructs, i.e. 2D matrix, 3D scaffold and hydrogel, were optimized as meaningful in vitro models that allow studying the influence of microenvironmental cues on MSC. A 2D decellularized cell-derived matrix was obtained by 10 or 21 days cultivation of human MSC followed by incubation with high-pH detergent solution and DNase. A 3D bioscaffold was produced by EDTA-based decellularization and decalcification of human femoral heads2. A translucent 3D hydrogel was obtained by pepsin digestion of decellularized bone powder followed by neutralization at 37°C for 1h. Removal of DNA was confirmed by nuclei absence observed on histological sections. Preservation of specific bone ECM-related proteins was analyzed by SDS-Page and immunostaining (particularly strong presence of preserved collagen-I was observed for all models). The ultrastructure of models was investigated by SEM and/or microCT, confirming preservation of the native ECM architecture. Resazurin assay and FACS analysis of recovered cells showed sustained MSC viability. Osteogenic differentiation seemed to be promoted by the models, as observed by alizarin-red staining and gene expression analysis. Future studies will investigate how the different complexity of models affects stemness and mechanosensing in MSC, providing insights in the influence of these microenvironmental cues on the regenerative potential of MSC.

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Abstract **#453**

FIBROBLAST-SEEDED LUNG EXTRACELLULAR MATRIX (ECM)-DERIVED HYDROGELS AS AN IN VITRO MODEL FOR STROMAL BED IN IDIOPATHIC PULMONARY FIBROSIS (IPF)

Mehmet Nizamoglu[P][1], Marco Schlepütz[2], Irene H. Heijink[1], Kaj E.C. Blokland[1], Matthew J. Thomas[2], Barbro N. Melgert[3], Janette K. Burgess[1]

[1] University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD (GRIAC), Department of Pathology and Medical Biology, Groningen, the Netherlands

[2] Immunology & Respiratory Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

[3] University of Groningen, Department of Molecular Pharmacology, Groningen Research Institute for Pharmacy, Groningen, the Netherlands

Corresponding author's email: m.nizamoglu@umcg.nl

Introduction:

Idiopathic Pulmonary Fibrosis (IPF) is characterized by aberrant extracellular matrix (ECM) deposition and remodeling, which orchestrates cellular responses to the fibrotic microenvironment[1]. Decellularized lung ECM-derived hydrogels resemble the mechanical properties[2] of native decellularized tissues, potentially providing a 3D model mimicking native cell-ECM interactions. We aimed to characterize this 3D human lung microenvironment model, with respect to stiffness and viscoelastic properties, in the presence and absence of primary human lung fibroblasts.

Materials & Methods:

Lyophilized powders of decellularized IPF and control lung matrices (pool of 6 patients) were pepsin digested, and formed to hydrogels seeded with control primary lung fibroblasts (n=4 donors), and cultured for 14 days. Stiffness and viscoelastic relaxation were measured by Low-Load Compression Testing[2] (20% strain).

Results:

IPF hydrogels were stiffer than controls (1.84 ± 0.33 kPA vs 1.37 ± 0.35 kPA), and became even more stiff when cell-seeded (1.91 ± 0.37 kPA) in contrast to controls which became softer (1.09 ± 0.27 kPA). Time to reach 100% viscoelastic relaxation was shorter in cell-seeded compared to native hydrogels for both IPF (19.14 ± 3.17 vs 41.6 ± 37.66 seconds) and control (11.44 ± 6.55 vs 22.21 ± 19.59 seconds).

Conclusion:

The mechanical properties of the ECM hydrogels were modified by fibroblasts, while in turn the ECM microenvironment altered cellular responses. These data suggest that higher stiffnesses and altered relaxation patterns of the ECM could contribute to the fibrotic response in IPF by instructing the cells. Fibroblast-seeded ECM-derived hydrogels can provide more insight on cell-ECM interactions in IPF.

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Abstract **#454**

USE OF TRANSCRIPTOMIC DATA FROM NON-WOUND HEALING PATIENTS TO PURPOSE IDENTIFIED GENES INVOLVED IN THE DISEASE.

Mansoureh Farhangniya[P][1,2], Ali Samadi kouchaksaraei[1], Farzaneh Mohamadi Farsani[3]

[1] *Department of Medical Biotechnology, Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Human and animal cells bank, Iranian Biological Resource Center, ACECR, Tehran, Iran.*

[3] *Department of Biology, faculty of science, NaghsheGahan University, Isfahan, Iran*

Corresponding author's email: m.farhangniya@gmail.com

The molecular mechanisms for tissue regeneration and wound healing are not yet well understood. Limited success of clinical trials indicates that a crucial aspect of the growth factor wound healing strategy is the effective delivery of these polypeptides to the wound site. A meta-analysis approach in which genetically clear the role of each gene and protein or signal pathway can help to overcome the limitations associated with the application of recombination growth factor proteins. Studies point to the synthesis of factors within the chronic wound environment that could have deleterious effects on the repair process. So, the understanding of how damage or loss of tissue can be reconstructed is one of the biggest challenges in biomedical research and facilitating reconstruction. In this paper, we use a bioinformatics approach and network theory for more accurately and better understanding the genetic underpinnings of wound healing mechanisms.We begin by selecting some literatures related genes in human wound healing in Gene Expression Omnibus to obtain the genes involved in this process and deduce the protein-protein interaction network from them. Then, we analyze the network to rank the genes associated with wound healing according to topological properties and find the hub genes among them. Finally, we perform a method to find the most important biological pathways in GO and KEGG. The findings, including gene expression analysis, confirm how a network-based bioinformatics method is able to classify candidate genes for analysis, thus accelerating the understanding of molecular mechanisms and assist for discovery of potential goals.

Keywords

wound healing; gene candidate; network analysis

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Abstract **#457**

AN INNOVATIVE, BIOMATERIAL-BASED DELIVERY SYSTEM FOR THE ANGIOGENIC GROWTH FACTOR VEGF

Matthias Schumacher$_P$[1], Debby Gawlitta[2], Pamela Habibovic[1], Sabine H van Rijt[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht, Netherlands*

[2] *Department of Oral and Maxillofacial Surgery & Special Dental Care, University Medical Center Utrecht, Netherlands*

Corresponding author's email: m.schumacher@maastrichtuniversity.nl

A limiting factor in clinical bone augmentation is sufficient neovascularization. A promising strategy to tackle this problem is the targeted release of pro-angiogenic growth factors (GFs) from the employed implant. One prominent example is the vascular endothelial GF (VEGF) that can enhance and pattern vascularization [1]. In this context, mesoporous bioactive glasses (MBGs) have been proposed as versatile GF carriers because of their intrinsic characteristics: a large surface area resulting from their highly ordered porosity on the mesoscale, as well as high degradability and bioactivity [2]. However, to date strategies to immobilize GFs on MBGs are solely based on electrostatic interactions – which are poorly controllable in terms of adsorption as well as release kinetics, and may deteriorate protein conformation and activity. Herein, we propose MBGs decorated with VEGF-binding peptide as novel biomaterial/GF-delivery system for bone augmentation applications. MBG was synthesized according to established protocols [2] and surface-functionalized with reactive amine groups using alkoxysilane chemistry [3]. Then, a peptide sequence known to specifically bind VEGF based on [4]) was covalently coupled to the amine functional groups. Both the degree of amine functionalization as well as the amount of immobilized binding peptide could be controlled. Finally, binding efficacy and specificity for VEGF as well as release kinetics and biological activity of the complexed GF were studied.

Taken together, the proposed system appears as promising new strategy to immobilize the pro-angiogenic factor VEGF on a highly biocompatible and bioactive biomaterial and deliver it into a bone defect without impairing its biological potency.

Keywords

mesoporous bioactive glass; growth factor delivery; bone

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Abstract **#458**

ZINC-MODIFIED SOL-GEL COATINGS: PROTEOMIC AND IN VITRO STUDY

Julio Suay[1], Francisco Romero-Gavilán[1], Andreia Cerqueira$_P$[1], Iñaki García-Arnáez[2], Ibón Iloro[3], Mikel Azkargorta[3], Félix Elortza[3], Mariló Gurruchaga[2], Isabel Goñi[2]

[1] Departamento de Ingeniería de Sistemas Industriales y Diseño, Universitat Jaume I, Castellón de la Plana, ES

[2] Facultad de Química, Universidad del País Vasco, San Sebastián, ES

[3] CicBiogune, Proteomics Platform, Derio, ES

Corresponding author's email: lages@uji.es

Zinc was described as the 'calcium of the twenty-first century' as consequence of its promising applications in bone tissue regeneration. This element can play an important role in stimulating the osteogenesis and mineralization and suppressing osteoclast differentiation [1]. In this study, new bioactive ZnCl2-doped sol-gel materials were designed to be applied as coatings onto titanium. The materials were synthetized through the sol-gel method using methyltrimethoxysilane and tetraethyl-orthosilicate as precursors and increasing amounts of ZnCl2. The coatings were physicochemically characterized through SEM, FTIR, NMR, hydrolytic degradation, Zn2+ release, contact angle and roughness measurements. Cytotoxicity, ALP activity, gene expression (ALP, RUNX2, RANKL, RANK, iNOS) in vitro testing were performed in MC3T3-E1 osteoblasts; and TNF-α, IL-1β, TGF-β and IL-4 gene expression and TNF-α cytokine secretion in RAW264.7 macrophages. The effect of Zn on the adsorption of human serum proteins onto the material surface was evaluated through nLC-MS/MS.Homogeneous coatings with a controlled Zn2+ release were obtained. The materials were no cytotoxic. Moreover, the addition of Zn increased the gene expression of ALP, TGF-β, and RUNX2 in the osteoblasts. A higher IL-1β, TGF-β, and IL-4 gene expression and a reduced TNF-α liberation were measured in macrophages. Proteomic results showed changes in the adsorption patterns of proteins related to immune, coagulative and regenerative functions, in a Zn dose-dependent manner. Overall, the developed coatings could improve bone regeneration capacity in a Zn dose-dependent manner, as well as exert an anti-inflammatory effect. Furthermore, a correlation between movements in protein adsorption patterns and cellular response was established.

Keywords

Hybrid sol-gel; Proteomics; zinc

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Abstract **#459**

CARTILAGE ORGANOID FORMATION FROM 3D CULTURE OF CHONDROCYTES AND NOTOCHORDAL CELL MATRIX

Meike Kleuskens[P][1], João Crispim[1], René van Donkelaar[1], Rob Janssen[1,2], Keita Ito[1]

[1] *Orthopaedic Biomechanics, Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands*

[2] *Orthopaedic Center Máxima, Máxima Medical Center Eindhoven/Veldhoven, The Netherlands*

Corresponding author's email: m.w.a.kleuskens@tue.nl

Autologous chondrocyte implantation is a clinically used technique to regenerate articular cartilage in focal defects, however it faces the challenge of chondrocyte dedifferentiation during 2D expansion in vitro. Previous research has shown that adding porcine notochordal cell matrix (NCM) to the culture medium induces proliferation and matrix production of bovine chondrocytes in alginate beads[1]. Besides, NCM is rich in proteoglycans and collagen type II, important cartilage matrix components. Therefore, it was hypothesized that adding NCM to a 3D chondrocyte culture provides the cells with a cartilage-like matrix to attach to, while also inducing proliferation. Healthy bovine and osteoarthritic human chondrocytes were cultured in spinner flasks (5*10^4 cells/ml) for 12 days with/without 1 mg/ml NCM. Adding NCM resulted in increased proliferation of the chondrocytes (5.6- and 3.9-fold for bovine and human, respectively) compared to the control group without NCM while viability of >90% was maintained throughout the culture period. It was observed that cells attached to the NCM as well as to each other. The organoids kept growing over time, reaching up to 500µm in diameter by day 12. Immunohistochemistry of NCM cultured organoids showed the presence of proteoglycans, collagen type II and VI, and cartilage-marker Sox-9, confirming the chondrogenic phenotype and the presence of an interterritorial and pericellular matrix within the organoids. This work shows the potential of using NCM supplementation in chondrocyte culture, as it leads to proliferation and organoid formation without compromising the phenotype of chondrocytes as in standard 2D expansion protocols.

Keywords

articular cartilage; chondrocyte expansion; notochordal cell matrix

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Abstract **#461**

REPAIR OF CHONDRAL DEFECTS WITH CARTILAGE ORGANOIDS IN HUMAN OSTEOCHONDRAL EXPLANTS

Meike Kleuskens[P][1], René van Donkelaar[1], Rob Janssen[1,2], Keita Ito[1]

[1] *Orthopaedic Biomechanics, Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands*

[2] *Orthopaedic Center Máxima, Máxima Medical Center Eindhoven/Veldhoven, The Netherlands*

Corresponding author's email: m.w.a.kleuskens@tue.nl

One of the persisting challenges in articular cartilage regeneration is the inferior quality of repair tissue and lack of integration with the surrounding cartilage, leading to suboptimal load distribution and degeneration of the surrounding cartilage. Culturing chondrocytes with porcine notochordal cell matrix (NCM) has been shown to form organoids containing collagen type II and proteoglycans. The present study hypothesizes that this might improve cartilage tissue formation and integration in short-term cartilage repair. In this study, this novel approach will be compared to an autologous chondrocyte implantation-like procedure using 2D expanded chondrocytes and Tisseel fibrin sealant. Chondrocytes derived from human tissue of patients undergoing total knee replacement (TKR) surgery (n=5) were expanded in spinner flasks supplemented with 1 mg/ml NCM, forming cartilage organoids, and in traditional 2D culture conditions for 12 days. Osteochondral explants (Ø 10mm, 5 per donor) were isolated from tissue of six different TKR subjects and a Ø 6mm chondral defect was created in the center of each explant. Explants were cultured in a double-chamber culture platform in which cartilage and bone compartments are separated to allow for supplementation of tissue-specific medium. Organoids and 2D cultured chondrocytes were implanted into the defects using Tisseel fibrin sealant (10^6 cells/80µl) and the constructs were cultured for 28 days. Characterization of the organoids in the spinner flasks before implantation shows the presence of proteoglycans, collagen type II and Sox-9. Analysis of the biochemical content as well as histological and immunohistochemical appearance of the cultured constructs after 28 days is pending.

Keywords

cartilage organoids; human osteochondral explant; chondral defect

Abstract **#462**

HOST RESPONSE AND MACROPHAGE ACTIVATION PROFILE TOWARDS MULBERRY AND NON-MULBERRY SILK

Guru Janani$_P$[1,2], Li Zhang[2], Stephen F. Badylak[2,3,4], Biman B. Mandal[1,5]

[1] *Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India*

[2] *McGowan Institute for Regenerative Medicine, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219-3110, USA*

[3] *Department of Surgery, School of Medicine, University of Pittsburgh, University of Pittsburgh Medical Center Presbyterian Hospital, 200 Lothrop Street, Pittsburgh, PA 15213, USA*

[4] *Department of Bioengineering, University of Pittsburgh, 3700 O'Hara Street, Pittsburgh, PA, 15261, USA*

[5] *Centre for Nanotechnology, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India*

Corresponding author's email: janani@iitg.ac.in

Mulberry (Bombyx mori, BM) and non-mulberry (Antheraea assamensis, AA) silk have been used in diverse tissue engineering applications, yet a detailed immune response towards these biomaterials is largely unknown. Here we present a detailed macrophage phenotype and host response towards BM, AA, and blend of BM and AA (BA) silk in comparison with extracellular matrix (ECM) scaffolds (liver ECM and small intestinal submucosa-SIS), and polypropylene (PP) mesh. Host remodeling events, including cell infiltration, macrophage activation, foreign body multinucleate giant cells (MNGC) response, vascularization, and collagen deposition have been evaluated following biomaterial implantation in rat partial-thickness abdominal wall defect model. AA and SIS elicited a pro-inflammatory response at 14 days that transitioned to an anti-inflammatory response by 35 days. All biomaterials showed higher cell infiltration on day 14, and BM, AA, and BA maintained higher cell infiltration at 35 days. LECM and SIS showed fewer MNGC compared to defect only and PP mesh, and AA showed significantly fewer MNGC than BM, BA, and PP mesh on 35 days. In addition, the activation profile of murine bone marrow-derived macrophages (BMDM) was assessed in vitro after treatment with degradation products of biomaterials. AA treatment stimulated a higher arginase:iNOS ratio compared to BM, BA, SIS, and pepsin in naive BMDM, and significantly increased Fizz1:iNOS ratio compared to BM, BA, LECM, SIS, and pepsin in pro-inflammatory challenged BMDM. The outcomes of the present study provide an improved understanding of macrophage-mediated events associated with the source of silk biomaterial, which would aid in tissue engineering applications.

Keywords

Silk ; Host response; Immunomodulation

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Abstract **#463**

DETERMINING THE MESH-SIZE AND DIFFUSION OF DYNAMIC SUPRAMOLECULAR HYDROGELS FOR OPTIMAL UREMIC TOXIN DIFFUSION IN TUBULAR EPITHELIAL CELLS.

Floor Ruiter[P][1,2], Jasia King[1], Nadia Roumans[1], Aurélie Carlier[1], Vanessa LaPointe[1], Matthew Baker[2]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Cell Biology-Inspired Tissue Engineering, Maastricht University, Maastricht, the Netherlands*

[2] *MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, Maastricht, the Netherlands*

Corresponding author's email: f.ruiter@maastrichtuniversity.nl

Due to its similarities to the natural extracellular matrix's structure and dynamic rearrangement, dynamic or supramolecular biomaterials have been of increased interest in the field of regenerative medicine. Although mesh-size and diffusion in fixed hydrogel networks are vastly investigated [1, 2], less research has focused on the effect of dynamic hydrogels on the diffusion of key nutrients and toxins.

Nutrient diffusion is important to maintain viable cells within the biomaterials. Moreover, when active cellular transport of molecules is required, this dynamicity should not interfere. This is for example important when these biomaterials are implemented in the design of tubular epithelial cell dialysis devices or kidney replacement (with organoids) for patients with chronic kidney disease[3]. With more than two million patients depending on dialysis with no possibility for kidney transplantation, many different strategies are investigated for the design of dialysis devices and organoid growth [4, 5] capable of toxin transport abilities, which conventional dialysis does not possess.

Here we sought the optimal mesh-size and diffusion properties by employing a variety of dynamic hydrogel systems with different mesh-size and cross-linking strategies. The diffusion coefficients for various protein sizes were determined. Renal tubular epithelial cells (sourced from adult stem cell derived 'tubuloids'[5]) were cultured on these hydrogels. The effect of the hydrogel's dynamicity on diffusion and cellular uptake of toxins were investigated to determine the required diffusion properties for optimal uremic toxin diffusion.

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Abstract **#464**

HARNESSING CIRCULATING MONOCYTES FOR IN SITU ENDOTHELIALIZATION OF BLOOD CONTACTING SUPRAMOLECULAR SURFACES

Dina Ibrahim$_P$[1], Moniek Schmitz[1], Paul Bartels[1], Anthal Smits[1], Patricia Dankers[1], Carlijn Bouten[1]

[1] *Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, the Netherlands*

Corresponding author's email: d.m.a.s.a.ibrahim@tue.nl

The lack of a hemocompatible blood-material interface represents a major challenge for implanted synthetic biomaterials that are in direct contact with blood [1]. The optimal way to meet such a pivotal hemocompatibility requirement is to create an endothelial lining at the implanted blood-material interface [2], ideally through in situ endothelialization. This requires i) implanting a biomaterial with specific characteristics to recruit and interact with circulating cells that can form a functional endothelium directly in the body [3], and ii) targeting the most favorable cell source for in situ endothelialization. The source of cells recruited in such a process has been under investigation, with the main focus on circulating endothelial progenitor cells (EPCs) [4,5]. However, EPCs represent a very small fraction of the peripheral blood (0.002%-0.01%) [6]. Growing evidence suggests that the more abundantly present monocytes (CD14+) have a strong potential to differentiate into endothelial-like cells, thereby contributing to endothelium formation on implanted artificial surfaces [7].
Here, we focus on designing and testing novel material surfaces to recruit and differentiate circulating monocytes for implant endothelialization. To this end, a supramolecular biomaterial based on hydrogen bonding units is functionalized via a modular approach [8,9] with a heparin binding peptide, to subsequently bind heparin and VEGF to the material. Current testing of the material in blood and under hemodynamic loading will tell the efficiency to recruit angiogenic monocytes and their ability to differentiate into EC like cells to form of a protective monolayer against thromboembolic events on blood-contacting supramolecular implants.

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Abstract **#465**

CO-CULTURES IN 3D PRINTED SCAFFOLDS FOR CARTILAGE REGENERATION FOR CRANIOFACIAL RECONSTRUCTION

Steven Posniak$_P$[1], Gordon Wallace[1], Johnson Chung[1], Xiao Liu[1]

[1] *University of Wollongong*

Corresponding author's email: sp695@uowmail.edu.au

INTRODUCTION:In the field of regenerative medicine, cartilage regeneration and craniofacial reconstruction remains a challenge as traditional methods are unable to adequately replicate the native cartilage tissue [1]. This study investigates an extrusion bioprinting approach to mimic and replace injured craniofacial cartilage.

METHODS:The 3D-BioplotterTM (EnvisionTEC, Gladbeck, Germany) was employed for bioprinting. Scaffolds were printed with the dimensions of 5mm*5mm and heights of 1mm. BM hMSCs were purchased from Lonza and primary chondrocytes were acquired from patients with consent. The hydrogel GelMA/HAMA was used with a ratio of 5:2 (w/v) containing 0.03% LAP. Scaffolds were printed with BM-hMSCs and chondrocytes at a density of 2 million cells/ml using BM-hMSC:Chondrocyte ratios of 1:0, 3:1, 1:1 and 0:1 through an 18G nozzle. Scaffolds were examined after 1-, 21- and 35 days. Photocuring was performed by utilizing 405nm visible blue light for 60 seconds. Scaffolds were characterized via immunostaining, DNA content analysis and qPCR.

DISCUSSION & CONCLUSIONS:MSCs and chondrocytes have numerous advantages and disadvantages when used for cartilage regeneration [2]. By combing these cell types, the disadvantages can be alleviated and produce an improved cartilage-like extracellular matrix. Additionally, this combination of bioprinting and cells successfully creates an environment that produces natively-similar chondrocytes which can potentially be used for craniofacial reconstructions.

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Keywords

Cartilage; Chondrogenesis; Bioprinting

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Abstract **#466**

BIOMICROMILL: A NEW NON-ENZYMATIC DEVICE FOR ENRICHMENT OF REGENERATIVE CELLS FROM ADIPOSE TISSUE

Marie-Sophie Narzt[1,2,3], Eleni Priglinger[1,2], Wurzer Christoph[1,2,4], Carolin Lindner[1,2], Karin Strohmeier[5], Michael Aufreiter[1,2], Susanne Suessner[6,2], Matthias Sandhofer[7], Susanne Wolbank[1,2], Heinz Redl[1,2,4], Marlene Wahlmüller[1,2,3]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Linz/Vienna, Austria*

[2] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[3] *MorphoMed GmbH, Vienna, Austria*

[4] *Liporegena GmbH, Breitenfurt, Austria*

[5] *School of Medical Engineering and Applied Social Sciences, University of Applied Sciences Upper Austria, Linz, Austria*

[6] *Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria*

[7] *Austrian Center for Lipedema, Linz/Vienna, Austria*

Corresponding author's email: marlene.wahlmueller@trauma.lbg.ac.at

Human adipose tissue is an attractive and abundantly available source of adult stem cells applicable in regenerative medicine and tissue engineering. Several companies developed systems aiming for a closed, sterile, safe and reproducible cell isolation process. However, many of these systems are based on enzymatic digestion with collagenase, which is the most expensive part of the isolation process, complicates regulatory authorization and may have negative impacts on cell potency and efficacy. The aim of this study was to test a newly developed non-enzymatic device called Biomicromill (BMM) that mechanically, in a non-enzymatic way homogenize the fat tissue for the enrichment of therapeutic cells for autologous clinical applications. Our new BMM device was compared to other non-enzymatic (shaking and cutting) and standard enzymatic isolation methods regarding cell yield, viability, ATP content and the presence of regenerative cells, subpopulations and differentiation potential into the adipogenic, osteogenic, chondrogenic and vasculogenic linage. After BMM isolation mature adipocytes were separated whereas regenerative cells were enriched regarding specific subpopulations such as endothelial progenitor cells (CD45-/CD31+/CD34+), pericyte-like cells (CD45-/CD31-/CD146+), and supra-adventitial adipose-derived stromal/stem cells (ASC) (CD45-/CD31-/CD146-/CD34+). BMM showed higher cellular ATP levels and stronger adipogenic, osteogenic and chondrogenic differentiation potential compared to cells isolated by enzymatic and the other tested non-enzymatic isolation methods. Cells derived by BMM showed the potential to form tube-like structures in a fibrin clot system. Here we tested a new cell enrichment device, which is able to reveal therapeutic cells of high quality without use of enzymes for immediate clinical application.

Keywords

stromal vascular fraction; adipose derived stromal/stem cells

Abstract **#467**

SPINAL CORD REPAIR: DO DENTAL STEM CELLS HAVE THE TEETH FOR IT?

Anne des Rieux[P][1], Pauline De Berdt[1], Laurent Gatto[2], Anibal Diogenes[3], Giulio Muccioli[1], Claudia Montero-Menei[4], Veronique Miron[5]

[1] *UCLouvain/LDRI*

[2] *UCLouvain/DDUV*

[3] *San Antonio University*

[4] *Angers University*

[5] *Edinburgh University*

Corresponding author's email: anne.desrieux@uclouvain.be

Our objective is to study the potential of human dental stem cells from the apical papilla (SCAP) as a therapeutic tool for the repair of spinal cord injury. SCAP are less studied than other MSC but our recent results indicate that they possess a yet non-explored therapeutic potential. Combined with the fact that they are easy to obtain, isolate and expand1, and in consequence cheap to produce, SCAP have a high translational potential.

We recently showed that SCAP decrease the expression of pro-inflammatory markers in activated microglia and support remyelination of spinal cord organotypic sections by protection of oligodendrocytes and stimulation of their progenitor differentiation, notably via the secretion of activin A2. We also observed that when seeded on pharmacologically active microcarriers (PAM)3 or subjected to a pro-inflammatory stimulus (unpublished data), SCAP expression of immunomodulatory molecules increased (PGE2, IDO-1, TSG-6 and IL10, activin A, TSG-6 and VEGF, respectively).

Injecting SCAP seeded on PAMs loaded with brain derived neurotrophic factor (BDNF) in a rat spinal cord contusion model3 or implanting a whole apical papilla in a rat hemisection model4 resulted in a significantly improved locomotor function. To further understand how SCAP could stimulate the repair of the spinal cord, RNAseq analysis is ongoing on human apical papillae retrieved 24h after their implantation in a rat spinal cord hemisection model.

Our research contributes to uncover the high therapeutic potential of SCAP and to better understand by which mechanisms they can stimulate tissue repair.

Keywords

spinal cord injury; dental stem cells; cell delivery

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Abstract **#468**

IN VITRO CO-CULTIVATION OF HUMAN CARTILAGE- AND BONE-LIKE MICROTISSUES

Annemarie Ecke[P][1], Erik Hentschel[1], Jenny Scholka[1], Anne-Helen Lutter[1,2], Ursula Anderer[1,2]

[1] Department of Cell Biology and Tissue Engineering, Institute of Biotechnology, Brandenburg University of Technology Cottbus-Senftenberg, Universitaetsplatz 1, 01968 Senftenberg

[2] Faculty of Health Sciences Brandenburg, Brandenburg University of Technology Cottbus-Senftenberg, Universitaetsplatz 1, 01968 Senftenberg

Corresponding author's email: Annemarie.Ecke@b-tu.de

Traumatic cartilage lesions can cause progressive degeneration of joint tissue and consequently osteoarthrosis. Current cell-based therapies mainly use chondrocytes to fill the defects without adding bone cells. The aim of this study was to evaluate the influence of osteoblasts on the differentiation of chondrocytes to develop a co-culture technique for optimal formation of the bone-cartilage part as transplant for deep cartilage lesions and possibly osteoarthrosis.

Human primary chondrocytes and osteoblasts isolated from knee joints were cultivated in vitro as 3D microtissues in cell-repellent plates for two weeks. For further two weeks, bone- and cartilage-like microtissues were co-cultured in close contact to one another. Cartilage-like microtissues alone served as differentiation control. The differentiation degree was evaluated via immunohistochemistry (IH) to detect bone- and cartilage-specific markers and histology to visualise typical glycosaminoglycans (Safranin O, Alcian blue) and calcium deposits (von Kossa) on cryosections.

As demonstrated by IH and histological analysis, expression of cartilage-specific markers as well as collagen type I was observed in cartilage-like microtissues after the first two weeks of culture. Co-cultured cartilage-like microtissues showed less collagen type I and II as well as Sox9 expression compared to cartilage-like microtissues cultivated alone. Bone-like microtissues in co-culture exhibited collagen type I and proteoglycan synthesis as well as calcium deposits.

This study suggests an influence of bone-like microtissues on collagen synthesis of cartilage-like microtissues by modulating Sox9 expression of chondrocytes. Further optimisations of the co-culture technique are necessary to improve the differentiation degree of both the bone and cartilage part of the microtissues.

Abstract **#469**

HUMAN EMBRYO-LIKE STRUCTURES AND CONCOMITANT ETHICAL AND POLICY CHALLENGES: AN EXPLORATIVE REVIEW

Ana M. Pereira Daoud[P][1,2,3], Wybo J. Dondorp[1,3,4], Annelien L. Bredenoord[2], Guido M. W. R. de Wert[1,3,4]

[1] *Department of Health Ethics and Society, Maastricht University, Maastricht, The Netherlands*

[2] *Department of Medical Humanities, Utrecht University Medical Center, Utrecht, The Netherlands*

[3] *School for Oncology and Developmental Biology (GROW), Maastricht University, Maastricht, The Netherlands*

[4] *School for Care and Public Health Research (CAPHRI), Maastricht University, Maastricht, The Netherlands*

Corresponding author's email: a.pereiradaoud@maastrichtuniversity.nl

Harnessing the potential of embryo-like structures (ELS) – i.e., 3D aggregates of pluripotent stem cells that resemble particular stages and/or regions of early (human) embryogenesis – whilst remaining within the parameters of responsible innovation will require mapping out the ethical and policy challenges raised by their research use. In so doing, this study identified challenges on three levels.

On a conceptual level, it is unclear whether human ELS qualify as human embryos. Large discrepancies across international definitions of the human embryo lead to equally discrepant positions regarding the conceptual qualification of ELS, either a priori ruling out the possibility of them qualifying as embryos or ultimately comprising epistemological uncertainty with regard to their qualification.

Equally undetermined is whether and the extent to which ELS can have moral status. On a normative level, the challenge thus becomes determining whether ELS could come to develop features of moral concern, how these features translate to specific biological markers, processes and structures, and the degree of moral value they confer. Deliberations of this kind are important to pursue in order to determine if and to what extent ELS research could present a morally preferable alternative to animal and/or human embryo research.

Finally, on a policy level, the challenge becomes developing policies capable of safeguarding against the particular intricacies of ELS research. The fact that scientists may be increasingly able to bypass morally relevant stages or events in ELS showcases the urgency of rethinking the foundational paradigms of contemporary ethical and legislative embryo protective frameworks.

Abstract **#470**

INTRAPERICARDIAL INJECTION OF HYDROGELS DERIVED FROM DECELLULARIZED CARDIAC EXTRACELLULAR MATRIX LOADED WITH MESENCHYMAL STROMAL CELLS AND THEIR SECRETOME: A NOVEL PROPOSAL OF THERAPEUTIC APPROACH TO CYTOSTATICS-INDUCED DILATED CARDIOMYOPATHY

Tácia T.A. Liguori$_P$[1,2], Gabriel R Liguori[1,2], Viktor Sinkunas[2], Cristiano J Correia[2], Raphael S.C. Silva[2], Fernando S Zanoni[2], Vera D Aiello[2], Martin C Harmsen[3], Luiz Felipe P Moreira[2]

[1] *TissueLabs SAGL*

[2] *Heart Institute (InCor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR*

[3] *Laboratory for Cardiovascular Regenerative Medicine Research (CAVAREM), Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands*

Corresponding author's email: gabriel@tissuelabs.com

INTRODUCTION: Intramyocardial injection (IM) of hydrogels containing stem cells, their secretome or both, may hold promise to treat dilated cardiomyopathy (DCM). This, however, may lead to adverse outcomes related to the trauma of the IM injection and poor conductibility of the biomaterial. Additionally, DCM is a multichambered disease, which demands a treatment setup that reaches the entire heart. We hypothesized that the intrapericardial injection of hydrogels derived from cardiac decellularized extracellular matrix (dECM) loaded with adipose tissue-derived stromal cells (ASC) and their secretome (conditioned medium, CMed) dampen or reverse the progression of DCM. METHODS: DCM was induced in rats through ten weekly intraperitoneal injections of doxorubicin (cumulative dose: 18mg/kg). In week five, the animals were divided in intrapericardial treatments (2ml/kg): 1) saline, 2) dECM hydrogel and 3) dECM hydrogel loaded with ASC and their CMed. ASC concentration was 20 million per mL while 100x concentrated CMed in hydrogel were used. Non-treated, healthy rats, were used as controls. Interstitial myocardial fibrosis was determined by Sirius Red and hemodynamic parameters were determined by pressure-volume loops. RESULTS: Interstitial myocardial fibrosis was reduced in ASC/CMed-treated animals compared to saline controls ($p=0.0139$). Ejection fraction and cardiac work efficiency were improved in the ASC/CMed-treated rats compared to saline ($p=0.0151$ and $p=0.0655$, respectively). Treatment with sole dECM hydrogel did not reduce fibrosis nor improve hemodynamic parameters. CONCLUSION: The intrapericardial injection of dECM hydrogels loaded with ASC and their secretome warrant a novel therapeutic possibility by improving ventricular hemodynamics and reducing cardiac remodeling in doxorubicin-induced DCM.

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Abstract **#471**

GLYCO-CONJUGATE BIOMATERIALS TO STUDY THE EFFECT OF EXTRACELLULAR MATRIX GLYCOSIGNATURE IN CELL FATE MODULATION

Francesca Cadamuro[P][1], Fabrizio Chiodo[2], Francesco Nicotra[1], Laura Russo[1]

[1] *Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy*

[2] *Department of Molecular Cell Biology and Immunology, Amsterdam Infection & Immunity Institute and Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam 1081 HV, The Netherlands*

Corresponding author's email: f.cadamuro@campus.unimib.it

Glycans play a key and dynamic role in regulating cellular functions and behaviour, including cell-cell and cell-ECM communication, signal transduction and molecular recognition. They are tethered to the extracellular matrix (ECM) components as poly- or oligosaccharides and on cell surface, where most of receptor are N- or O-glycosylated. Many cell development events, including cell adhesion, growth, migration, differentiation, host-pathogen interactions are associated with changes in oligosaccharide structures.[1][2] The study of differential ECM glycosignature effect is limited today by the lack of glyco-tools able to resemble both, glycan and proteins identities. Here in this work a panel of differential glycosylations were developed on collagen type I and gelatin, in order to characterize the effect of both glycosignature and ECM proteins motifs. The biomolecular interaction of glycoconjugate ECM mimetics were tested on solid-phase assays for the interaction with Siglec-9, Siglec-10 and DC-SIGN, carbohydrate-binding proteins expressed at extracellular level and with immunomodulatory functions. Preliminary studies on soluble and 3D structured glycoconjugates polymers were performed to validate a combinatorial platform to study and tailor cell fate for tissue engineering and in vitro cultures.

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Abstract **#472**

TOWARDS BIOLOGICALLY-INSPIRED AND COMPUTATIONALLY-DESIGNED HUMAN CARDIAC ENGINEERED TISSUES USING MELT ELECTROWRITING AND HIPSCS

Manuel M. Mazo Vega[P][1], Pilar Montero[1], María Flandes-Iparraguirre[1], Konstantinos A Mountris[2], Ana S. de la Nava[3], Saioa Musquiz[1], Andreu Climent[3], Estefanía Peña[2], Esther Pueyo[2], Manuel Doblaré[2], Francisco Fernández-Avilés[3], Felipe Prósper[1]

[1] *Cima Universidad de Navarra, Clínica Universidad de Navarra and Idisna, Pamplona, Spain*

[2] *Aragón Institute of Engineering Research, University of Zaragoza, IIS Aragón Zaragoza, Spain and CIBER-BBN, Spain*

[3] *Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Universidad Complutense, Madrid, Spain. CIBERCV, ISCIII, Madrid, Spain*

Corresponding author's email: mmazoveg@unav.es

Introduction: Successful biomimetism requires exquisite design of engineered tissues. Fibre-reinforcement of hydrogels is able to overcome their inherent mechanical weakness, but introduces cues, affecting the functional outcome, especially in high-anisotropy tissues as the myocardium. Here, we generate human cardiac tissues, and apply computational modelling to optimize fibre reinforcement for the desired functionality.

Methods: Uniaxial and biaxial mechanical tests were employed. hiPSC-cardiomyocytes (CMs) were embedded in matrigel, casted on MEW-PCL fibres and cultured for 28 days. Gene-expression, structure (IF) and functionality (optical mapping) were assessed and compared with conventionally-cultured hiPSC-CMs in 2D. This information was used to generate computational electrophysiological models to investigate the impact of the MEW-PCL fibres on the activation pattern of hiPSC-CMs.

Results: Fibre mechanics dominated the properties of the composites. hiPSC-CMs formed microtissues and were able to survive and contract for 4 weeks. Gene expression highlighted an increased maturity, with fibres aligning CMs, which redounded in faster conduction velocities. In silico simulations revealed a high impact of the composite's pore size on the velocity of electrical propagation. Maximum local activation time (LAT) was 51 ms and increased up to 71 ms for pore sizes of 100 µm and 700 µm. Simulation of 2D-cultured hiPSC-CMs led to maximum LAT values of 350 ms due to the loose coupling of the cardiomyocytes.

Conclusion: Composite systems matured, at structural, gene-expression and functional levels. Simulations provided insight on the impact of the MEW-fibres on the activation pattern of hiPSC-CMs, and open the way for the fabrication of tailored human cardiac tissues.

Abstract **#473**

A BIOINSPIRED TEMPLATE FOR ADVANCED IN VITRO BONE REMODELING MODELS

Bregje W. M. de Wildt$_P$[1], Anat Akiva[2], Nico A.J.M. Sommerdijk[3], Keita Ito[1], Sandra Hofmann[1]

[1] Orthopaedic Biomechanics, Department of Biomedical Engineering and Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven, The Netherlands.

[2] Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

[3] Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.

Corresponding author's email: b.w.m.d.wildt@tue.nl

Human in vitro bone remodeling models can potentially facilitate the investigation of physiological bone remodeling while addressing the principle of replacement, reduction and refinement of animal experiments (3R). Physiological bone remodeling follows a specific sequence of events: activation, bone resorption by osteoclasts, reversal, and bone formation by osteoblasts [1]. Current in vitro models typically use osteoblast-osteoclast cocultures to study cell-cell interactions in two dimensions. As such, cell-matrix interactions and their temporal organization are often neglected. To enable the investigation of cell-matrix interactions and to mimic the sequence of these interactions in vitro, a bone-mimetic template is required [1, 2]. Here, we aimed at developing such a template, inspired by bone's extracellular matrix composition and organization. Using silk-fibroin as an organic matrix, poly-aspartic acid (pAsp) to mimic the functionality of non-collagenous proteins [3], and 10x simulated body fluid as mineralization medium [4], we were able to prepare mineralized silk fibroin films and scaffolds. By using pAsp in the mineralization medium, mineral precipitation in the medium was inhibited and mineralization of the organic matrix (both films and scaffolds) was enhanced. The surface of the mineralized films and scaffolds was covered with a layer of mineral crystals. This mineralization led to an improved young's modulus both at the nanometer and millimeter scale. After cell seeding, scaffold remodeling was tracked with micro-computed tomography over a period of 6 weeks. The use of this template for in vitro bone remodeling models enables the investigation of cell-matrix interactions and their sequence, characteristic for physiological bone remodeling.

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Abstract **#474**

MECHANICAL CONSTRAINTS FOR ECM REORGANIZATION IN A 3D IN VITRO MYOCARDIAL INFARCTION MODEL

Ignasi Jorba$_P$[1], Dylan Mostert[1], Carlijn Bouten[1]

[1] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands - Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, the Netherlands.*

Corresponding author's email: i.jorba.masdeu@tue.nl

After myocardial infarction (MI) tissue suffers from a massive loss of cardiomyocytes (CMs), together with a loss of extracellular matrix (ECM) organization, leading to an impairment of the coordinated contraction. Current approaches for myocardial regeneration mainly focus on enhancing the number of contractile cardiomyocytes to the damaged area, without taking into account the restoration of ECM anisotropic organization. Recent findings show that ECM organization can be controlled by the mechanical environment, such as cyclic strain. Here, by using a 3D cardiac model developed previously in our laboratory [1], we propose that changing on demand the strain pattern of the tissue will induce ECM remodeling to anisotropic organization. A co-culture of iPSC-CM and primary cardiac fibroblast will be seed in a collagen I and Matrigel hydrogel to mimic the biological, biochemical environment of MI tissue. By locally modifying the stiffness of the hydrogel we will alter the strain pattern of the beating tissue. The platform is based on flexible micropillars that allows tissue contraction force measurements. Additionally, the platform is compatible with confocal microscopy to visualize ECM remodeling and cell orientation. The results of the study will contribute to design strategies to reorganize ECM after MI.

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Abstract **#475**

CHONDROGENICALLY PRIMED MESENCHYMAL STEM CELLS ARE ABLE TO FORM BONE IN THE PRESENCE OF AN ALLOGENEIC HUMAN IMMUNE SYSTEM

Eric Farrell[P][1], Virginia Palomares Cabeza[1,2], Niamh Fahy[1], Shorouk Fahmy Garcia[1], Janneke Witte-Bouma[1], Pieter Brama[2], Martin Hoogduijn[3]

[1] *Department of Oral and Maxillofacial Surgery, Erasmus Medical Centre, Rotterdam, The Netherlands*

[2] *School of Veterinary Medicine, University College Dublin, Dublin, Ireland*

[3] *Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands*

Corresponding author's email: e.farrell@erasmusmc.nl

Chondrogenically primed mesenchymal stem cells (MSCs) have been shown to form bone in vivo through the endochondral ossification pathway, representing a potential off- the-shelf allogeneic option for bone repair. The aim of this study is to investigate the ability of chondrogenically primed paediatric MSCs (pMSCs) to form bone in vivo and determine its persistence within an allogeneic immune system.21 day chondrogenically differentiated human pMSCs were subcutaneously implanted in immunodeficient IL2Rγ-/-RAG2-/- mice. Mice received an intraperitoneal injection of allogeneic human Peripheral Mononuclear Blood Cells (PBMCs) 3 weeks before implantation or 8 weeks after. uCT scans were performed at either week 3, or 8 and 12 post-implantation. Blood, bone constructs and femurs were analysed. No significant differences in the volumes of the bone constructs were detected at weeks 8 and 12. Human T cell infiltration was observed in the constructs at weeks 3 and 12 post-implantation, with a predominance of the CD4 subtype at week 3 and of the CD8 at week 12. On this time point, bone-forming cells of human and mouse origin were identified. No significant changes in the levels of circulating human CD45 cells were detected in the blood of the humanized animals. Our results show that bone derived from chondrogenically primed pMSCs can persist in the presence of a mismatched immune system without the development of significant immune responses. In this process cells from host and donor sources play a role. These findings illustrate the potential of pMSC-derived bone constructs for clinical use in an allogeneic setting.

Keywords

allogeneic mesenchymal stem cells; bone repair; non-immunogenic

Abstract **#476**

3D BIOPRINTING OF THE MEDIA LAYER OF SMALL-CALIBER BLOOD VESSELS WITH A DECELLULARIZED ARTERIAL EXTRACELLULAR MATRIX-BASED BIOINK

Gabriel R Liguori[1,2], Viktor Sinkunas[2], Tácia T.A. Liguori[1,2], Emerson G Moretto[1,3], Martin C Harmsen[4], Luiza Felipe P Moreira[2]

[1] TissueLabs SAGL

[2] Heart Institute (InCor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR

[3] Departamento de Engenharia de Sistemas Eletrônicos, Escola Politécnica, Universidade de São Paulo, SP, Brazil

[4] Laboratory for Cardiovascular Regenerative Medicine Research (CAVAREM), Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands

Corresponding author's email: gabriel@tissuelabs.com

INTRODUCTION: 3D bioprinted vascular grafts represent a promising alternative for the replacement of small-caliber blood vessels (SCBV). We evaluated the use of a vascular decellularized extracellular matrix (dECM) based bioink as a biomaterial for 3D bioprinting of the tunica media of SCBV. METHODS: Vascular (aortic) dECM-based solution was used in the present study. Upon warming to 37ºC, the solution turns into a hydrogel. Differentiation of ASC to smooth muscle cells (SMC) on the hydrogel was induced with 10ng/mL of TGF-β1 and SM22α used as a differentiation marker. A combination of the hydrogel and 10 million cells/mL was used as bioink for 3D bioprinting of the tunica media layer of SCBV in an extrusion-based 3D bioprinter using the freeform reversible embedding of suspended hydrogels (FRESH) method. The morphology of the 3D bioprinted tissue construct was evaluated after 24 hours, 7 days, and 21 days with Masson's trichrome staining. RESULTS: The hydrogel drove spontaneous (without TGF-β1) differentiation of ASC to SMC. The staining of 3D bioprinted SCBV evidenced the maturation of the construct along the time. At 24 hours, ECM hydrogel containing cells shows a porous pattern, becoming more compact the longer the construct is cultured. At 21 days, a well defined compact cell-rich vessel wall characterizes the construct. CONCLUSION: Bioinks based on vascular dECM hydrogels offer cells a microenvironment mimetic to the native tissue, spontaneously differentiating ASC to SMC, and supporting 3D bioprinting of the tunica media layer of SCBV, representing a promising alternative for the manufacturing of vascular grafts.

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Abstract **#477**

INHIBITION OF THE TYROSINE KINASE RECEPTOR EPHA2 DECREASES HYPERTROPHY AND INFLAMMATION OF CHONDROCYTES AND MESENCHYMAL STEM CELLS

Mauricio N Ferrao Blanco[P][1], Roberto Narcisi[1], Fjodor Bekedam[1], Yvonne Bastiaansen-Jenniskens[1], Andrew A Pitsillides[2], Gerjo J.V.M. van Osch.[1]

[1] *Erasmus MC, University Medical Center, Rotterdam, NL*

[2] *The Royal Veterinary College, University of London, UK*

Corresponding author's email: r.narcisi@erasmusmc.nl

Inflammation and hypertrophic differentiation are key processes that dampen stable hyaline cartilage development from mesenchymal stem cells (MSCs). Accumulating data suggest these processes are also involved in the progression of osteoarthritis, leading to the modification and degradation of cartilage extracellular matrix. Therefore, the aim of this study was to identify a novel target for osteoarthritis (OA) and cartilage tissue engineering, associated with chondrocyte hypertrophy and inflammation.

To identify targets, we combined previously published microarray data sets from both mice and human origin [1-2-3]. Combination of datasets revealed candidate targets, among which the Ephrin receptor A2 (EphA2), a tyrosine kinase receptor, was the only new target identified.

EphA2 was found to be upregulated in the hypertrophic compared to the proliferative zone of the murine growth plate and in human OA versus healthy cartilage. In chondrogenically-differentiated human MSCs, the EphA2 activity inhibitor ALW-41-27 dose-dependently reduced the hypertrophic markers COL10A1, ALPL, IHH and the catabolic enzymes MMP3 and MMP13. Additionally, EphA2 inhibition did not influence glycosaminoglycan nor Collagen-II deposition, suggesting that the effect of ALW-41-27 was mainly targeting the hypertrophic differentiation. Similarly, in OA chondrocytes, ALW-41-27 reduced the expression of COL10A1, MMP3 and MMP13, but also significantly decreased the production of nitric oxide and IL-6 when the chondrocytes were stimulated with the inflammatory factor TNFα.

These results identify EphA2 to be associated with osteoarthritic cartilage, chondrocyte hypertrophy and inflammation. Based on our data, ALW-41-27 may constitute a new approach for OA treatment by limiting hypertrophy and inflammatory pathways in resident articular cartilage chondrocytes.

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Abstract **#478**

BIFUNCTIONAL SCAFFOLDS FOR ADIPOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS AND PHOTOTHERMAL ABLATION OF BREAST CANCER CELLS

Guoping Chen[P][1,2], Xiuhui Wang[1,2], Naoki Kawazoe[1]

[1] *Research Center for Functional Materials, National Institute for Materials Science, Japan*

[2] *Department of Materials Science and Engineering, Graduate School of Pure and Applied Sciences, University of Tsukuba, Japan*

Corresponding author's email: Guoping.CHEN@nims.go.jp

Breast cancer is a major public health issue, whose morbidity and mortality are increasing across the world. Surgical resection is the most widely used and preferred therapy in clinic. However, surgical resection cannot completely eliminate all the cancer cells and may result in cancer metastases and recurrence. Moreover, surgical resection is always accompanied with large breast defects that are difficult to self-heal. It is strongly desired to design a bifunctional material for effective breast cancer therapy and breast reconstruction. In this study, bifunctional composite porous scaffolds of gold nanorods (AuNRs) and gelatin with well controlled pore structures were prepared by introducing AuNRs into the porous matrices of gelatin and using ice particulates as a porogen material. The composite scaffolds had large spherical pores with good interconnectivity, which were beneficial to cell adhesion and distribution in the scaffolds. The composite scaffolds showed high photothermal conversion effect, whose photothermal temperature could be modulated by the amount of incorporated AuNRs, NIR laser power intensity and irradiation time. The composite scaffolds exhibited excellent photothermal ablation capacity of breast tumor cells in vitro and in vivo. When hMSCs were cultured in the cmposite scaffolds, the cells adhered and proliferated throughout the scaffolds. The composite scaffolds enhanced formation of lipid vacuoles and expression of adipogenic genes (CEBPA, PPARG, LPL, FABP4 and FASN). The results indicated the AuNRs-gelatin composite scaffolds had a bifunctional property for simultaneous photothermal ablation of breast cancer cells and adipogenic differentiation of hMSCs.

Abstract **#479**

THE RESPONSE OF PRIMARY HUMAN MACROPHAGES TO DECELLULARIZED NASAL SEPTAL CARTILAGE

David Gvaramia$_P$[1], Johann Kern[1], Yvonne Jakob[1], Hanna Tritschler[2], Rolf E. Brenner[2], Roman Breiter[3], Julia Kzhyshkowska[4], Nicole Rotter[1]

[1] Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, Department of Otorhinolaryngology, Head and Neck Surgery, Mannheim, Germany

[2] Division for Biochemistry of Joint and Connective Tissue Diseases, Department of Orthopaedics, University of Ulm, Ulm, Germany

[3] Institute of Bioprocess Engineering, University of Erlangen, Germany

[4] Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, Germany

Corresponding author's email: David.Gvaramia@medma.uni-heidelberg.de

As key regulators of host immune response, macrophages can determine the success of biomaterial transplantation. Macrophages are responsible not only for the inflammatory reaction by M1 but also for the process of constructive remodeling of degradable biomaterials by M2 macrophages. Here we used an in vitro approach to evaluate the polarization of primary human monocyte-derived macrophages (MDM) into M1 or M2 in response to the porcine decellularized nasal septal cartilage (DNSC).

MDM were cultured in the presence of DNSC, or two types of porcine-derived biomaterials for reference. The response of MDM was monitored during a 6-day culture period by microscopy and flow cytometry, as well as for secretion and gene expression of inflammatory factors.

Initially, DNSC induced an M1-like inflammatory response, as seen by the increased production of TNF-α, IL-1β, and IL-6 by MDM. By the end of the culture period, the secretion of M1-factors reduced, while production of the M2-specific cytokine, CCL18 increased. Furthermore, the increase of the M2-specific marker, CD206 was observed on DNSC. Overall, we observed a mixed reaction of MDM to the DNSC. However, the initial pro-inflammatory reaction could be effectively antagonized by IL-4. Moreover, preloading of DNSC with IL-4 markedly reduced the production of M1 factors and induced an early polarization of MDM towards M2, as evidenced by the increase in CCL18 gene expression.

Overall, DNSC induced a mixed M1/M2 response in MDM. However, the functionalization of DNSC with IL-4 effectively antagonized the M1 component of the immune response.

Keywords

Decellularized Matrix; Cartilage; Macrophages

Abstract **#480**

SILVER NANOPARTICLES-LOADED POLY(VINYL ALCOHOL)/CHITOSAN/GRAPHENE HYDROGELS OBTAINED BY ELECTROCHEMICAL SYNTHESIS

Katarina Nešović[1], Ana Janković[1], Rade Surudžić[1], Maja Vukašinović-Sekulić[1], Vesna Kojić[2], Tamara Radetić[1], Vesna Mišković-Stanković[P1]

[1] *University of Belgrade, Faculty of Technology and Metallurgy, 11000 Belgrade, Serbia*

[2] *University of Novi Sad, Faculty of Medicine, Oncology Institute of Vojvodina, 21204 Sremska Kamenica, Serbia*

Corresponding author's email: vesna@tmf.bg.ac.rs

Recent advances in biomaterials science are increasingly focused on (re)search for next-generation soft tissue regeneration solutions. Hydrogel-based materials are becoming a focal point to address many tissue engineering requirements, possessing exceptional biocompatibility, with tailorable shape, structure and mechanical properties, as well as infinite possibilities drug delivery. The widespread antibiotics use has led to aggravated resistance issues in many bacterial strains, so the focus is increasingly shifted towards alternative antibacterial solutions such as silver nanoparticles (AgNPs). In this work, we have developed novel composite materials based on poly(vinyl alcohol)/chitosan (PVA/CHI) and poly(vinyl alcohol)/chitosan/graphene (PVA/CHI/Gr) hydrogels with silver nanoparticles obtained through green in situ constant-voltage electrochemical synthesis. The obtained AgNPs' properties, sizes and size distributions were examined using UV-visible spectroscopy, dynamic light scattering and transmission electron microscopy. In vitro swelling and silver release kinetics were monitored in the simulated physiological conditions (pH7.4, 37 °C). The obtained release and swelling isotherms were fitted with several theoretical models that helped discern the mechanisms of these processes. Tensile testing was carried out to examine the composites' mechanical behavior and elasticity – important properties for soft tissue engineering materials. Finally, the excellent antibacterial activity of the obtained silver/poly(vinyl alcohol)/chitosan (Ag/PVA/CHI) and silver/poly(vinyl alcohol)/chitosan/graphene (Ag/PVA/CHI/Gr) hydrogels against Staphylococcus aureus and Escherichia coli was confirmed quantitatively by colony counting method, and their non-toxicity was verified towards two model fibroblast cell lines (MRC-5 and L929) utilizing the MTT assay. Through thorough characterization, the obtained nanocomposite hydrogel materials were identified as strong candidates for active antibacterial soft tissue engineering materials.

Abstract **#481**

DUAL-“CLICK” BIOINKS FOR THE 3D BIOPRINTING OF IN VITRO MINI-JOINT MODELS

Pierre Tournier$_P$[1], Nathan Lagneau[1], Boris Halgand[2], François Loll[1], Jérôme Guicheux[2], Catherine Le Visage[1], Vianney Delplace[1]

[1] Université de Nantes, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

[2] Université de Nantes, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

Corresponding author's email: pierre.tournier@univ-nantes.fr

Osteoarthritis (OA) is the most common debilitating joint disease, and its treatments remains mainly symptomatic. There is a tremendous need for the study of the onset, progression and treatment of OA through in vitro joint disease models, of particular interest for drug screening prior to undertake preclinical studies. To this end, 3D bioprinting allows the custom creation of biomimetic structures using cell-laden hydrogels as bioinks. However, bioinks tend to be mechanically weak and collapse after extrusion.

In this work, we developed an injectable hyaluronic acid (HA)-based hydrogel using an innovative, dual dynamic/non-dynamic covalent crosslinking strategy. By varying the molecular weight (100-300 kDa) and concentration (1-3% (w/v)) of HA, we successfully obtained 3D printable stable viscoelastic hydrogels with a large range of mechanical properties (80 to 1000 Pa for G’ at 1 Hz). Using L929, a murine fibroblastic cell line, we demonstrated the excellent cytocompatibility of the newly designed viscoelastic hydrogels, with a high proportion of viable cells (95% after two days), and an increased metabolic activity and DNA content over time, suggesting a cell proliferation. The chondrogenic differentiation of human mesenchymal stromal cells (hMSCs) encapsulated in these gels was further evaluated over 28 days. The gene expression of a panel of chondrogenic markers, including type II-collagen, aggrecan and SOX9, was evaluated by real-time PCR, followed by the histological analysis of a glycosaminoglycan-rich extracellular matrix.

Together, our results highlight the potential of a new class of injectable hydrogels to bioprint OA models and drive chondrogenic commitment of hMSCs in vitro.

Abstract **#482**

GRAPHENE-LOADED BIOACTIVE HYDROXYAPATITE COATINGS ON TITANIUM SUBSTRATE - FUNDAMENTAL IN VITRO INVESTIGATIONS

Milena Stevanović[1], Marija Djošić[2], Ana Janković[1], Katarina Nešović[1], Vesna Mišković-Stanković[1]

[1] *University of Belgrade, Faculty of Technology and Metallurgy, 11000 Belgrade, Serbia*

[2] *Institute for Technology of Nuclear and Other Mineral Raw Materials, 11000 Belgrade, Serbia*

Corresponding author's email: ajankovic@tmf.bg.ac.rs

There is an increasing demand for the development of safe, reliable, and long-lasting orthopedic devices for skeletal tissue engineering. Hydroxyapatite (HAP) has lately gained a lot of attention as a coating material for bone implants. Poor mechanical and antibacterial properties of HAP can be surpassed by introducing other components to the composite – graphene (Gr) and silver. Graphene is expected to improve the coatings' mechanical properties and to ensure a better interconnected composite structure.Well-known antibacterial effect of silver in bacterial infections treatments makes it a logical choice for composite coating production. In this work, the electrophoretic deposition (EPD) technique was successfully used for obtaining HAP and biocomposite HAP/Gr, Ag/HAP, and Ag/HAP/Gr coatings on Ti plates. Single-step cataphoretic EPD was performed from ethanolic suspensions. Obtained coatings exhibited uniform surface without cracks, as demonstrated by FE-SEM analysis. Elastic, plastic, and fracture response of coatings' surfaces was investigated using the nanoindentation test. HAP/Gr and Ag/HAP/Gr coatings exhibited higher hardness and lower penetration depths i.e. they were more resistant to indentation as opposed to their counterparts without graphene. Substantial improvement was achieved and values of mean reduced elastic modulus were increased up to 50 %. Antibacterial activity was monitored against Staphylococcus aureus and Escherichia coli bacterial strains and pointed to the strong antibacterial capacity of the silver-containing coatings (Ag/HAP and Ag/HAP/Gr) within first 3h post-incubation. Based on these results, Ag/HAP/Gr composite coating electrodeposited on titanium exhibited strong antibacterial properties and favorable mechanical properties making it a promising candidate for bone implant materials development.

Abstract **#483**

ULTRALOW AMOUNT OF ENGINEERED PLANT VIRAL NANOPARTICLES ATTACHED ON MESENCHYMAL STEM CELLS ENHANCES OSTEOGENESIS AND MINERALIZATION

Ying-Ying Lin[P][1], Juliane Schuphan[2], Christina Dickmeis[2], Eva Miriam Buhl[3], Ulrich Commandeur[2], Horst Fischer[1]

[1] *Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany*

[2] *Institute for Molecular Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany*

[3] *Electron Microscopy Facility, Institute of Pathology, RWTH Aachen University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany*

Corresponding author's email: ylin@ukaachen.de

Potato virus X (PVX), a filamentous biocompatible plant viral nanoparticle with more than 1200 potential sites for targeted molecule exposition, is a novel nanocomposite for hydrogel-based materials, which often require functional molecules to mimic extracellular matrix in bone tissue engineering. Here, PVX was genetically modified to precisely present highly concentrated mineralization- and osteogenesis-associated peptides in a local area, individually. These engineered viral nanoparticles (VNPs) were laden in agarose or agarose-collagen blended hydrogels at a concentration lower than other studies by two orders of magnitude. The effectiveness of enriched peptides on VNPs was proven and compared with free peptides and VNPs presenting only 10% and 30% functional peptides. Osteogenic induction analyses of mesenchymal stem cells, including alkaline phosphatase activity, Alizarin red staining and gene expression, showed significant increases when under the stimulation of VNPs. An attachment of VNP to cells was observed under confocal microscopy, illustrating a stimulation of biomimetic peptides in close proximity of the cells. The release of VNPs in hydrogels was quantified and more than 80% of VNPs remained inside after several culturing procedures. Cell containing VNP-laden hydrogel solutions were further used to develop a bone substitute via drop-on-demand bioprinting technology. Printing repeatability was high, demonstrating a suitable solution as a bioink. Cell viability and cell-VNP distribution in bioprinted hydrogels were monitored with fluorescence microscopy and 2-photon laser scanning microscopy, respectively, and osteogenesis was once again evaluated. Overall, this study demonstrated that PVX nanoparticles are excellent candidates for hydrogel nanocomposites in bioink development and bone tissue engineering.

Keywords

plant virus nanoparticles; biomineralization; bone tissue engineering

Abstract **#485**

THE HUMAN FETAL AND ADULT STEM CELL SECRETOME CAN COUNTERACT DETRIMENTAL OXIDATIVE STRESS IN A PRECLINICAL MOUSE MODEL OF DOXORUBICIN-INDUCED CARDIOTOXICITY

Federico Villaᵖ[1], Ambra Costa[2], Silvia Bruno[2], Mingchuan Li[3], Michele Russo[3], James Cimino[3], Clarissa Ruggeri[1], Rodolfo Quarto[4], Pietro Ameri[1], Alessandra Ghigo[3], Silvia Ravera[2], Bollini Sveva[2], Roberta Tasso[2]

[1] *IRCCS Ospedale Policlinico San Martino, Genova, Italy*

[2] *Department of Experimental Medicine, University of Genova, Genova, Italy.*

[3] *Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy*

[4] *Department of Experimental Medicine, University of Genova, Genova, Italy*

Corresponding author's email: sveva.bollini@unige.it

INTRODUCTION

Cardiovascular side effects are major drawbacks of oncological drugs, like doxorubicin (Dox), as leading to late onset cardiomyopathy. Dox can impact activity of cardiomyocyte mitochondria, causing oxidative stress (1). We showed that the human fetal amniotic fluid-stem cell (hAFS)-conditioned medium (hAFS-CM) exerts remarkable pro-survival effects on Dox-exposed cardiomyocytes in vitro (2). Here we compare hAFS and human adult adipose tissue mesenchymal stromal cells (hMSC) paracrine potential in a Dox-derived cardiotoxicity murine model.

METHODS

hAFS were isolated from left over amniotic fluid of diagnostic amniocenteses; hMSC were obtained from liposuction aspirates. hAFS and hMSC were preconditioned under hypoxia (1% O2) for 24h to enrich their cell-conditioned medium (CM). A Dox-cardiotoxicity mouse model (3) was employed to investigate long-term hAFS-CM and hMSC-CM effects. Functional activity of cardiomyocyte mitochondria was also evaluated via in vitro confocal and biochemical analyses.

RESULTS

hAFS-CM and hMSC-CM inhibited body weight loss and improved myocardial function in the cardiotoxicity mouse model ($p<0.01$). The treatment with hAFS-CM and hMSC-CM partially reverts the impairment of mitochondrial complex I activity, oxygen consumption, and ATP synthesis induced by Dox ($p<0.0001$). Moreover, both treatments recover about 25% of the dysfunction of catalase and glutathione reductase ($p<0.001$), determining a reduction of lipid peroxidation. hAFS-CM and hMSC-CM preserved mitochondria organization and metabolism in injured cardiomyocytes ($p<0.0001$).

CONCLUSIONS

Both hAFS and hMSC can be appealing paracrine source to be exploited for preserving cardiomyocyte mitochondria function. Further analyses are ongoing to profile molecular candidate(s) in their secretomes to define the optimal formulation for future therapeutic strategies.

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Abstract **#486**

CAPTURING THE BIOPHYSICAL CUES OF THE VASCULARISED BONE OSTEOID EXTRACELLULAR MATRIX THROUGH THIOL-ENE PHOTO-CLICK CHEMISTRY

Laurens Parmentier[P1], Peter Dubruel[1], Sandra Van Vlierberghe[1,2]

[1] *Polymer Chemistry and Biomaterials Group – Centre of Macromolecular Chemistry (CMaC) – Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281, S4-Bis, 9000 Ghent, Belgium*

[2] *Brussels Photonics – Department of Applied Physics and Photonics, Vrije Universiteit Brussel and Flanders Make, Pleinlaan 2, 1050 Brussels, Belgium*

Corresponding author's email: laurens.parmentier@ugent.be

Four million surgeries are performed annually to treat bone defects using either bone grafts or synthetic substitutes rendering it the second most transplanted tissue worldwide [1]. Given the drawbacks associated with auto- and allografts, synthetic scaffolds mimicking the composition, architecture and properties of the native extracellular matrix are gaining increasing interest [2]. These biophysical cues are hereby converted into biochemical cues through mechanosensitive pathways that regulate cell behaviour [3]. The establishment of vascularisation however is one of the current challenges especially in critically sized bone defects [2,4]. Therefore, the aim is to develop a biomimetic hydrogel scaffold mimicking the bone osteoid in which proper vasculature is induced. Thiol-ene step-growth photocrosslinking was herein selected to enable superior control, homogeneous network formation and lower radical concentrations compared to traditional chain-growth systems [5]. More specifically, norbornene-functionalised gelatin/hyaluronic acid systems were combined with thiolated gelatin to function as cell-interactive crosslinker thereby forming networks with mechanical properties capable of triggering either osteo- or angiogenesis. The modification degree of these systems was characterised through proton nuclear magnetic resonance spectroscopy and an ortho-phthalic dialdehyde assay. Furthermore, in-situ photo-rheology, swelling and gel fraction experiments were used to evaluate the obtained networks. Moreover, in vitro quantification of angiogenesis was performed through a tube formation assay using human umbilical vein endothelial cells. Finally, osteogenesis was quantified through the production of alkaline phosphatase, the presence of calcium deposits and osteogenic gene expression of encapsulated adipose-derived stem cells. The results showed that networks could be created which can stimulate angiogenesis and osteogenesis.

Keywords

Osteogenesis; Angiogenesis; Thiol-ene crosslinking

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Abstract **#487**

THE DISTORTION OF MEW PATTERNS BY 3D COLLECTORS: INVESTIGATION, QUANTIFICATION AND CORRECTION

Cathal D O'Connell$_P$[1,2], Natasha Antill-O'Brien[2], Stephanie Doyle[1,2], Carmine Onofrillo[3,2], Serena Duchi[3,2], Claudia Di Bella[3,2]

[1] *School of Engineering, RMIT University, Melbourne, VIC, Australia*

[2] *BioFab3D, Aikenhead Centre for Medical Discovery, St Vincent's Hospital Melbourne, VIC, Australia*

[3] *Dept Surgery, University of Melbourne, VIC, Australia*

Corresponding author's email: cathal.o'connell2@rmit.edu.au

Integrating melt-electrowriting (MEW) with other fabrication technologies, such as extrusion-printing or bioprinting, offers tantalising possibilities for multi-scale bio-manufacturing.[1] However, to fully realise these goals, we must overcome new challenges arising when performing MEW upon 3D, rather than 2D, collectors.[2]
While planar or mandril collectors induce an electrohydrodynamic acceleration only in the vertical direction, 3D-collectors produce an additional, horizontal, force pulling fibers towards the 3D-object. Here, we report our investigation of how this force distorts MEW patterns around and atop 3D collectors.

As a model system, we used hemispherical collectors (various sizes and compositions) placed upon a conducting 2D-substrate. We quantified deviation of fibers from their intended positions as a function of (i) distance, r, from the hemisphere, (ii) the surface area, SA, of the hemisphere, (iii) the nozzle height, h. We found that the horizontal acceleration, and therefore the horizontal force, is directly proportional to: 1/r^5, SA^2 and h^2 respectively. In light of these data, we consider the physical origins of the horizonal acceleration, concluding that the distortion force is likely of dielectrophoretic, rather than an electrophoretic, origin.

Following this, we derived a simple model which estimates fiber deviation in such systems, and we use an optimisation algorithm to calculate tool-paths which account for these deviations. Finally, we validated how g-code produced by this algorithm effectively corrects the distortion effect, restoring our ability to create well-defined MEW patterns near and atop hemispherical collectors.

This study is a step towards understanding the complexities of MEW on non-planar collectors, towards multitechnology biofabrication.

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Abstract **#488**

ELECTROSPINNING AND CHARACTERISATION OF A BIOMIMETIC POLYMER SCAFFOLD CONSISTING OF ISOCYANATE-FREE POLYURETHANE AND COLLAGEN TYPE I FOR CARDIAC IMPLANTS

Dmitri Visser$_P$[1], Hadi Bakhshi[2], Ellena Hönig[1], Wolfdietrich Meyer[2], Katja Schenke-Layland[1,3,4], Hanna Hartmann[1]

[1] NMI Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen, 72770 Reutlingen, Germany

[2] Fraunhofer-Institut für Angewandte Polymerforschung IAP, 14476 Potsdam, Germany

[3] Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, 72076 Tübingen, Germany

[4] Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Corresponding author's email: dmitri.visser@nmi.de

Due to the rising numbers of people diagnosed with heart insufficiency, the clinical relevance of the development of new long-lasting and reliable biomimetic materials for functional heart implants is higher than ever. Native porcine pericardium currently acts as the gold standard despite the drawbacks of the intensive decellularisation processes involved therein. To provide an alternative that mimics the non-linear mechanical properties of porcine pericardium, we aim at developing a novel biomimetic electrospun hybrid consisting of polyurethane and collagen as part of a cardiac support system for patients with left ventricular heart failure.

Therefore, high molecular weight isocyanate-free polyurethanes (Mw = 14.000 - 26.000 g/mol) were synthesized by the transurethanisation reaction of dimethylhexane-1,6-diyldicarbamate and polycarbonate diols. The chemical structures of the polymers were characterized by NMR and FTIR spectroscopy, DSC and GPC. Consecutively, they were evaluated on their biocompatibility and electrospun into fibrous scaffolds with fibre diameters in the submicron range, as shown by SEM analysis.

Collagen could be successfully dissolved in gentle solvents and used to establish an electrospinning process with native murine collagen Type I. Morphological analysis was performed with SEM, after which a quality control based on SDS-PAGE and non-invasive and non-destructive imaging methods, such as Raman was deployed to assess whether collagen can be processed without denaturation. The latter is especially relevant, as the impact of conventional electrospinning solvents on the structural integrity of collagen is controversially debated.[1]

Keywords

collagen; polyurethane; Raman

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Abstract **#489**

BIODEGRADABLE LAMINARIN MICROGELS FOR BIOMOLECULES RELEASE

Edgar Castanheira[P][1], Tiago R. Correia[1], João M. M. Rodrigues[1], João F. Mano[1]

[1] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro*

Corresponding author's email: e.castanheira@ua.pt

Fabrication of polymeric matrixes for controlled release have been extensively explored for tissue engineering applications. Systems based on biopolymers exceed conventional polymers in biocompatibility, biodegradability and cost efficiency[1]. Thus, natural polysaccharides are commonly used in tissue engineering due to their biological properties. Biodegradable carriers based on bioactive marine polysaccharides have been reported, however these materials are often based on charged or high molecular weight (HMw) biopolymers[2]. Hence, HMw polymers creates higher density matrixes, diminishing nutrient diffusion, while charged polymers may establish unspecific interactions. Herein, we introduce biodegradable and biocompatible microgels synthesized from laminarin, a low Mw marine polysaccharide with great biological activity (e.g. immune modulation). Within this work[3], controlled size microgels were synthesized from novel modifications to laminarin through a click chemistry mild process that do not interfere in living cells encapsulation. Furthermore, microgels has a controlled release, achieving a burst of 40% release after 24h and a continuous release (7% per day) over 5 days. Additionally, full degradability of the microgels in physiological conditions was achieved after 11 days. Live/Dead assays have shown no significant cellular death after 24h to concentrations up to 100 µg/mL. Moreover, with an assessment of morphology, no membrane and neither nucleus seemed to have been disturbed. Our results indicate that the developed biodegradable microgels are biocompatible when in culture with hASCs while presenting a controlled release. Concluding these biomaterials are promising platforms for cellular differentiation due to easy incorporation of biomolecules (e.g. dexamethasone), full degradability and sustained release over a long period of time.

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Abstract **#490**

CONTROLLING BLOOD COAGULATION IN SUPRAMOLECULAR VASCULAR ACCESS GRAFTS VIA FEEDBACK-RESPONSE MECHANISMS

Boris Arts[P][1], Patricia Dankers[1]

[1] *Eindhoven University of Technology*

Corresponding author's email: b.arts@tue.nl

Currently 1 in 1000 people in Europe suffer from end-stage renal disease and require hemodialysis[1]. Hemodialysis is a medical procedure to remove waste products from the blood where the blood circulation of the patient is directly connected to a dialysis machine, often, via a vascular access graft (VAG). The primary failure of VAGs are due to its low patency rates (the time the graft remains open), primarily caused by thrombosis due to poor hemocompatible properties of the material. Several studies showed that coating the inner lining of the graft, e.g. with heparin, improves the patency rate[2,3]. However major concerns are expressed regarding the long-term efficacy of those coatings. Hereto the aim of this study is to improve the hemocompatibility of these grafts by controlling blood coagulation through a feedback-response mechanism.

Here we make use of bisurea (BU)-based supramolecular polymers. BU motifs can self-assemble via hydrogen bonding resulting in dynamic crosslinks embedded in a soft amorphous polymer phase. Bioactive molecules, e.g. peptides, can be functionalized with BU and mixed in through a modular approach to implement specific properties in the material[4].

In our approach heparin is conjugated to the surface of our VAG model materials via a BU-modified thrombin cleavable peptide (TCP). When in contact with whole blood the enzyme thrombin is designed to cleave the peptide causing heparin to be released (response). In turn heparin can form an inhibitory complex with anti-thrombin, naturally present in blood, thereby inhibiting thrombin activity and decreasing heparin release (feedback).

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Abstract **#491**

TWIST1 REGULATES CELLULAR SENESCENCE IN MESENCHYMAL STEM CELLS

Chantal Voskamp$_P$[1], Laura A Anderson[1], Wendy J.L.M. Koevoet[1], Sander Barnhoorn[1], Pier G Mastroberardino[1], Gerjo J.V.M. van Osch.[1], Roberto Narcisi[1]

[1] *Erasmus MC, University Medical Center, Rotterdam, NL*

Corresponding author's email: r.narcisi@erasmusmc.nl

Mesenchymal stem cells (MSCs) are promising cells to treat skeletal related diseases because of their (multi)differentiation capacity. However, one of the limitations that reduces their therapeutic use is cellular senescence, a state in which cells stop proliferating, while still metabolically active. By acquiring a specific senescence-associated secretory phenotype (SASP), senescent MSCs can contribute to tissue degeneration and chronic inflammation, also via the modulation of cellular metabolism. The transcription factor TWIST1 is suggested to play a role in cellular senescence, since its expression is reduced in slow proliferating MSCs and we previously demonstrated that high TWIST1-expressing MSCs have an enhanced expansion capacity [1-2]. The aim of this research is therefore to determine the role of TWIST1 in regulating cellular senescence and cellular metabolism of MSCs.

Here, we demonstrated that silencing TWIST1 in MSCs increased the occurrence of cellular senescence (siTWIST1-induced senescent MSCs). These siTWIST1-induced senescent MSCs had a different SASP profile compared to irradiation-induced senescent MSCs, and characterized by lacking of IL8 and IL6 expression. In addition, metabolic evaluation performed by the Seahorse XF apparatus showed that both siTWIST1-induced senescent and irradiation-induced senescent MSCs had a higher oxygen consumption rate compared to control cells, while siTWIST1-induced senescent cells had a lower extracellular acidification rate compared to the irradiation-induced senescent MSCs. Overall, our data indicate how TWIST1 regulation influences senescence in human MSCs, highlight the importance of TWIST1 as potential target for tissue engineering approaches and show that siTWIST1-induced senescent MSCs are characterized by a specific SASP and metabolic state.

Keywords

Senescence; Cellular metabolism; TWIST1

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Abstract **#492**

MOTILE BIOACTIVE EMBEDDING BATHS BASED ON BIOMATERIAL HYDROGEL PARTICLES.

Vasileios Trikalitis[P][1], Fabian Stein[1], Julia Perea-Paizal[2,3], Jeroen Rouwkema[1]

[1] Department of Engineering Technology, TechMed Institute, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands.

[2] 2 Department of Bioengineering, Imperial College London, South Kensington Campus, London SW72AZ, United Kingdom

[3] 3 Dynamical Cell Systems Laboratory. Chester Beatty Laboratories, Division of Cancer Biology. Institute of Cancer Research, London SW3 6JB, United Kingdom

Corresponding author's email: v.trikalitis@utwente.nl

Motile bioactive Embedding baths based on biomaterial hydrogel particles.

Wet hydrogel supensions have been used as 3D bioprinting support baths in order to bypass the challenges emerging from non-optimal rheological properties of bioinks(1-2). The materials used as suspensions, were usually chosen based on their ability to behave as low yield stress fluids, in order to achieve high quality printing within them(3).However hydrogel suspensions consisting of hydrogel microparticles, are a class of metamaterials on their own accord, and thus can offer a wide variety of options for tissue engineering by allowing the cells to interact with the suspension(4–7).

We fabricated granular metamaterials from popular hydrogel formulations, namely Alginate, GelMa, and Collagen-Agarose. The transition from uniform hydrogel constructs to wet granular suspensions of particles in the range of 50-200µm, enabled embedded 3D printing. We assessed the different microparticles as embedding baths by printing within them, and asessed their bioactivity compared to their continuous hydrogel counterpart.

When optimized, the suspensions allowed smooth muscle cells to manipulate the macro-shape of the embedding bath during culture. By testing different cell concentrations of Smooth Muscle Cells (SMC) and Human umbilical vein endothelial cells we observed that the change of the macroshape was also significantly affected.

Materials with this property to change their shape driven by cell contractility-based compaction, have been called kinomorphs(8) and thus we have discovered a way to induce kinesiomorphia to conventional biomaterials.

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Abstract **#493**

OSTEOGENIC DIFFERENTIATION AND TISSUE FORMATION OF HUMAN MESENCHYMAL STROMAL CELLS ON ADDITIVE MANUFACTURED BIODEGRADABLE COMPOSITE BIOMATERIALS FOR NON-LOAD BEARING CRANIO-MAXILLOFACIAL BONE REGENERATION

David Koper[P][1,2,3], Jiaping Li[1,2], Martyna Nikody[1,2], Peter Kessler[3], Pamela Habibovic[1], Lorenzo Moroni[2]

[1] Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

[2] Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

[3] Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Center, Maastricht, the Netherlands

Corresponding author's email: d.koper@maastrichtuniversity.nl

Surgical repair of skull bone defects is often performed using patient-specific polymeric or metallic implants.(1) These implants, however, have poor osseointegration ability, and their life-long fixation is dependent on osteosynthesis screws.(2) We developed alternative porous implants using additive manufacturing (AM), combining a biodegradable polymer with an osteoconductive calcium phosphate ceramic. In this study, we have examined the osteogenic differentiation and tissue formation capacity of bone marrow-derived human mesenchymal stromal cells (hMSCs) on these implants. Porous poly(ethyleneoxide terephthalate)/poly(butylene terephthalate) (1000PEOT70PBT30) 3D scaffolds without or containing 30, 40 or 50 weight% beta-tricalcium phosphate (TCP), produced using 3D fiber deposition, were used. The scaffolds were seeded with hMSCs and cultured in basic or mineralization medium. On days 1, 14 and 28, the constructs were analyzed for DNA content, tissue formation, alkaline phosphatase (ALP) activity, and osteopontin and osteocalcin secretion. hMSCs cultured on the scaffolds showed similar cell growth on all scaffold types. hMSCs produced ALP, osteopontin and osteocalcin. However, we observed minimal tissue formation throughout the porous scaffolds in all conditions after 28 days. We did not detect differences in hMSCs osteogenic differentiation on the scaffolds upon increasing TCP content. This might be due to limited degradation of the polymer carrier in vitro, resulting in limited exposure of the TCP phase to the cells, which is required to support MSCs osteogenic differentiation.(3) In the near future, we plan to further validate the scaffolds using hMSCs and investigate the effects of adding TCP using in vivo models.

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Abstract **#494**

DEVELOPMENT OF FULLY-POLYMERIC ELECTRIC LEADS FOR BIOELECTRONICS

Alexey Novikov$_P$[1], Josef Goding[1], Rylie A. Green[1]

[1] *Department of Bioengineering, Imperial College London, London, SW7 2BP, UK*

Corresponding author's email: alexey.novikov17@imperial.ac.uk

The use of metals as electric leads in bioelectronics, such as pacemaker leads, can be a common point of device failure (1,2). Conductive elastomers (CE), composites of conductive polymers (CP) and elastomers, are a promising alternative for bioelectronic applications. Their capacity to be soft, flexible and MRI compatible make them an exciting avenue for the fabrication of fully-polymeric electric leads.In this study, gel-based CEs were explored for the fabrication of electric leads. Gel-CEs consist of a CP gel system embedded in an elastomeric matrix. The gel forms continuous conductive pathways throughout the CE composite. This enables use of low CP contents, helping to preserve the mechanical properties of elastomeric matrix. Thus, a fabrication route for a gel-CE 1mm diameter wire was developed. First, poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) was thermally gelled into a hydrogel which was subsequently lyophilised twice. The aerogel was then embedded inside a matrix of solvent-casted polydimethylsiloxane or radically polymerised polyacrylamide. Scanning electron microscopy/energy-dispersive X-ray spectroscopy (SEM/EDS) confirmed the inclusion of continuous PEDOT:PSS network within the matrices. SEM study showed that the second lyophilisation step increased gel's pore size, which is deemed as a major factor in development of a uniform matrix. However, occasional pores were observed in the material. Overall, the study has shown the feasibility of reproducible gel-CE wire fabrication with different types of matrices. Thus, the method potentially offers a whole group of mechanically robust and electroactive materials for small-scale bioelectronics, which properties can be specifically tailored with gel and matrix materials choice and modification.

Keywords

Conductive elastomer; Fullly-polymeric bioelectronics; Gel-based composite

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Abstract **#495**

DESIGN AND INVESTIGATION OF AN IN VITRO 3D GLIOBLASTOMA PERIVASCULAR NICHE

Rosalyn Hatlen$_P$[1], Padma Rajagopalan[1]

[1] *Virginia Tech*

Corresponding author's email: rrhatlen@vt.edu

Glioblastoma (GBM) is the most deadly form of brain cancer, with a median survival time of 15 months1. One migration mechanism suggested for GBM cells is movement along blood vessels, in an area labelled the perivascular niche (PVN)1. Investigating how GBM and endothelial cells interact in the PVN may provide a deeper understanding of this cancer.

Our goal was to design a 3D in vitro model of the PVN. We assembled hydrogels composed of Type 1 collagen or a hyaluronic acid-gelatin mixture (Hystem-C®). While collagen is not prevalent in brain tissue, it is present in GBM tumors and can be relevant within elastic moduli similar to the brain. Since hyaluronic acid is found in the brain matrix, Hystem-C® mimics this chemical composition1.

Layered 3D hydrogels were prepared in glass-bottom tissue culture well plates. First, a thin collagen gel containing fluorescent beads was deposited, followed by a layer of collagen I or Hystem-C®. After gelation, a fibronectin-coated coverslip of LN229 (human glioblastoma) or human umbilical vein endothelial cells (HUVECs) was placed atop the stacked gels. For co-cultures, the second cell type was seeded around the coverslip.

HUVECs were cultured up to 14 days. HUVECs began to form connections around day 7 and by days 10-14, 3D connectivity was observed. LN229 migrated through the gel by day 6. We are currently investigating changes in migration and directionality in mono- and co-cultures of these cells. In the future, we aim to include macrophages and astrocytes to obtain a comprehensive understanding of the PVN.

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Abstract **#497**

OSTEOGENIC DIFFERENTIATION AND TISSUE FORMATION OF HUMAN MESENCHYMAL STROMAL CELLS ON ADDITIVE MANUFACTURED TITANIUM ALLOY-CALCIUM PHOSPHATE CERAMIC SCAFFOLDS FOR CRANIOPLASTY

David Koper[1,2,3], Jiaping Li[1,2], Martyna Nikody[1,2], Peter Kessler[3], Lorenzo Moroni[2], Pamela Habibovic[1]

[1] Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

[2] Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

[3] Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Center, Maastricht, the Netherlands

Corresponding author's email: d.koper@maastrichtuniversity.nl

Titanium (Ti) and its alloys have a long history as biomaterials for skull bone repair.(1) However, Ti (alloys) generally do not allow firm osseointegration.(2) Bioactive coatings can be applied, but it is difficult to obtain a homogenous coating.(3) We recently developed 3D porous implants from a Ti alloy and a CaP ceramic using additive manufacturing. This study analyzed the capacity of bone marrow-derived human mesenchymal stromal cells (hMSCs) towards osteogenic differentiation and tissue formation on these implants. Porous Ti6Al4V 3D scaffolds without or containing 5 or 10 weight% beta-tricalcium phosphate (TCP) were used. Upon culturing hMSCs on the different scaffolds for 14 or 28 days in basic or mineralization medium, cell metabolic activity, DNA content, tissue formation, alkaline phosphatase (ALP) activity, and osteopontin and osteocalcin secretion were analyzed. hMSCs cultured on the scaffolds remained metabolically active and showed a similar proliferation profile on all scaffold types. hMSCs produced ALP, osteopontin and osteocalcin. Additionally, we observed tissue formation throughout the porous scaffolds in all conditions after 28 days. No significant differences in ALP activity or osteocalcin and osteopontin secretion of hMSCs and tissue formation were observed between scaffolds without and with different amounts of TCP. This is in contrast with previous studies, showing that the addition of CaP typically increases the levels of ALP activity, osteocalcin and osteopontin.(4) Further in vitro studies as well as in vivo testing of the Ti-TCP scaffolds will be used to better understand the effect of the individual components and structural characteristics on the biological response.

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Abstract **#498**

BIOMIMETIC AND NANOSTRUCTURED COATINGS TO ENHANCE THE INTEGRATION OF HERNIA MESH IN THE ABDOMINAL WALL

Chiara Tonda-Turo[P][1], Giulia Giuntoli[1], Giuliana Muzio[2], Chiara Actis[2], Alessandro Ganora[3], Stefano Calzone[3], Matteo Bruno[3], Gianluca Ciardelli[1], Irene Carmagnola[1]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy*

[2] *Department of Clinical and Biological Sciences, University of Torino, Italy*

[3] *Dipromed Medical Devices S.r.l, San Mauro Torinese, Turin, Italy*

Corresponding author's email: chiara.tondaturo@polito.it

Abdominal hernia is one of the most common conditions worldwide and the use of prosthetic meshes is now largely accepted as gold standard of the surgical strategy. Mesh design is a crucial point to define the properties and surgical outcomes of the implant1. The choice of mesh materials and manufacturing methods influence mesh biocompatibility, mechanical properties, porosity, and degradation rates. This work aims to develop a multicomponent hernia mesh device able to enhance the integration of the polypropylene (PP)-based hernia mesh thanks to the presence of a nanostructured biomimetic substrate able to improve cell attachment and new tissue formation. A commercial polypropylene (PP) hernia mesh was coated with a nanofibrous membrane based on polycaprolactone (PCL) and gelatin (Gel) through the electrospinning technique. Resulting PCL-Gel nanofibers were homogeneous and defect-free, with an average diameter of 0.15±0.04 μm, and well retained their morphology even after 28 days of incubation in physiological conditions. The presence of Gel decreased PCL hydrophobicity, while it slightly influenced mechanical properties. The biocompatibility of the coated PP meshes was assessed by seeding and culturing BJ human fibroblasts on the device, up to 7 days. After 24 h, cells adhered to the nanofibrous substrate, and after 72h their metabolic activity significantly increased demonstrating cells proliferation. The absence of detectable lactate dehydrogenase in the culture medium indicated that no necrosis induction occurred. Hence, the developed nanostructured coating provided the PP mesh with chemical and topographical cues similar to the native extracellular matrix thus enhancing mesh integration in the abdominal wall.

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Abstract **#499**

HARNESSING MICROFLUIDIC BIOFABRICATION AND CELL PRINTING TO DEVELOP AN IN VITRO BONE PAIN MODEL

Gianluca Cidonio[P][1,2], Federico Salaris[1], Janos Kanczler[2], Alessandro Soloperto[1], Chiara Scognamiglio[1], Andrea Barbetta[3], Jonathan Dawson[2], Richard Oreffo[2], Alessandro Rosa[1,4], Giancarlo Ruocco[1]

[1] *Center for Life Nano Science, Italian Institute of Technology, Viale Regina Elena 291, 00161 Rome, Italy*

[2] *Bone and Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton, United Kingdom*

[3] *Department of Chemistry, Sapienza University of Rome, 00185 Rome, Italy*

[4] *Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy*

Corresponding author's email: gianluca.cidonio@iit.it

3D bone models remain of significant interest for the study of diseases associated with the skeletal system. Current approaches seek to use living organisms as bioreactors, however these do not address the 3Rs initiative. We detail a novel animal-free three-dimensional model for drug screening against bone pain, printing human bone marrow stromal cells (hBMSCs) and induced pluripotent stem cell-derived Nociceptors (iPSC-NCs) to fabricate a 3D platform to study the pathophysiological interaction of these cells.

An innovative clay-based biomaterial ink (bone ink), as previously established [1], was used to print hBMSCs. A low-polymeric fraction gelatin methacryloyl – Matrigel (GelMA-Ma) ink (neuro ink) was developed and characterised for the printing of the iPSC-derived nociceptors. Extrusion of the bone and neuro inks was tested on polydimethylsiloxane (PDMS) flow-focusing microfluidic chip using high-speed imaging (Photron-UX100) to determine the ideal extrusion parameters at 5 and 8 µl·min-1, respectively. Harnessing the ability of the microfluidic chip to rapidly switch between the bone and the neuro bioinks, hBMSCs and iPSC-NCs were printed serially, which critically demonstrated viability and functionality after printing, staining positively for alkaline phosphatase and expressing TRKA and CGRP, with limited expression of NEFH and RET. Significantly, the mechanical disruption of the neuro portion impaired functional transmission of neuro-signalling, confirming the ability to imitate the neural damage.

In conclusion, we demonstrate the functionality of a bone-neuro model able to recapitulate the complex skeletal innervation and to simulate /modulate the neural network, illustrating the potential of the model using novel therapeutic agents for bone pain studies.

Keywords

Pain; Bone ; Nerve damage

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Abstract **#500**

ADIPOGENIC DIFFERENTIATION OF ADIPOSE STEM CELLS ON SILK FIBROIN SPONGE SCAFFOLDS

Jana M. Keimes$_P$[1], Justus P. Beier[1], Tim Ruhl[1], Rebekka Götzl[1]

[1] *Department of Plastic Surgery, Hand and Burn Surgery, University Hospital RWTH Aachen, Aachen, Germany*

Corresponding author's email: jkeimes@ukaachen.de

The regeneration and replacement of soft tissue and research of appropriate donor tissues is increasingly important in an aging society. In this in vitro project the adipogenic differentiation of human adipose stem cells (ASCs) on sponge scaffolds made of silk fibroin was investigated to examine the optimal differentiation process for producing a fat tissue graft.
Human ASC were seeded with a number of 2x105 cells in 100 µl medium on fibroin sponge scaffolds and were stimulated towards adipogenic differentiation using 1 ml differentiation media based on High Glucose Medium supplemented by various concentrations of FBS (0%, 1% and 2%) and either rosiglitazone (5,6 µM) or Cremophor® EL (300 µM). On days 0, 3, 5, 7, 10 and 14 the Presto-Blue conversion indicated cellular vitality. The media supernatants were collected after 10 and 14 days and were analysed by ELISA. The scaffolds were cryosectioned and taken for histological staining methods. A 2D plate culture served as control.
Presto-Blue increased over the course of the experiment, indicating cell proliferation. Furthermore, ELISA indicated increased secretion of adiponectin and leptin. Adipogenically differentiated cells were also detected by immunohistochemical staining. We found 1% FBS and Cremophor® EL is the optimal culture setting.
Our experiments showed that silk fibroin sponge as a scaffold for an adipogenic differentiated cell culture can be produced by the addition of 1% FBS and Cremophor® EL, which could enable implantation of fatty tissue grafts in vivo.

Keywords

Adipogenic Differentiation; Adipose Stem Cells; Silk Fibroin Sponge Scaffolds

Abstract **#501**

INTRINSICALLY CROSS-LINKED, GLYCOSAMINOGLYCANS MULTILAYERS: A TOOL TO CONTROL CELLULAR BEHAVIOR & BMP-2 DELIVERY FOR BONE REGENERATION

Reema Anouz$_P$[1], Tamaradobra Selekere[1], Adrian Hautmann[1], Catharina Husteden[2], Christian Wölk[2], Matthias Menzel[3], Christian Schmelzer[3], Thomas Groth[1,2,4]

[1] *Biomedical Materials Department, Martin Luther University Halle-Wittenberg, Halle, Germany*

[2] *Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Halle, Germany*

[3] *Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Halle, Germany*

[4] *Interdisciplinary Center of Materials Science & Interdisciplinary Center of Applied Science, Martin Luther University Halle-Wittenberg, Halle, Germany*

Corresponding author's email: reema.anouz@student.uni-halle.de

This study aims to fabricate extracellular-like multilayers using glycosaminoglycans with capability to bind BMP-2 specifically in order to control osteogenic differentiation of cells by biocompatible release systems.

Native chondroitin sulfate (CS) and its oxidized form as polyanions were combined with collagen I as polycation to form multilayer coatings on model materials with getting advantage of the intrinsic cross-linking formed between oxidized CS and Col I to improve multilayer stability and affect the release of BMP-2. 50:50 mixture of native and oxidized CS was also studied. The myoblast cell line C2C12, which can differentiate into osteoblasts was seeded on 10 µg/mL BMP-2 loaded multilayers. Cell adhesion, osteogenic differentiation and BMP-2 release were investigated.

C2C12 cells cultured on the multilayers showed that BMP-2 loaded, oxidized CS multilayers promoted an osteogenic differentiation that was even higher than the positive control, when 10 µg/mL BMP-2 was added directly to the medium. Interestingly, the BMP-2 had synergistic effect on cell adhesion and spreading. BMP-2 in oxidized CS multilayers was successfully loaded to the layers, sustainably released over time and affected cell differentiation more than the soluble BMP-2.

The results show that oxidized glycosaminoglycans multilayers are useful as reservoirs for BMP-2* in which the intrinsic cross-linking affected BMP-2 release, improved multilayers stability due to the resulting stiff surface compared to the native ones, supported cell adhesion and subsequent differentiation. This can pave the way for coating implants and scaffolds for repair and regeneration of bone fractures.

This work was supported by the International Graduate School AGRIPOLY.

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Abstract **#502**

DEVELOPMENT OF A 3D-PRINTED IN VITRO-VESSEL MODEL FOR THE IMPROVEMENT OF EFFICACY OF STEM CELL THERAPIES FOR MUSCULAR DYSTROPHIES

Mattis Wachendörfer[P][1], Florence van Tienen[2], Hubert Smeets[2], Horst Fischer[1]

[1] *Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany*

[2] *Clinical genomics, Faculty of Health, Medicine and Life Sciences, Maastricht University, Universiteitssingel 50, 6229 ER Maastricht, The Netherlands*

Corresponding author's email: mwachendoerf@ukaachen.de

Muscular dystrophies are a group of genetic diseases characterized by progressive skeletal muscle weakness and degeneration. The intra-arterial injection of in vitro cultured mesoangioblasts, mesenchymal cells located on the vessel walls, holds potential for an effective stem cell therapy. The mesoangioblasts extravasate from the vessel through the vessel walls into the surrounding muscles, where they form healthy muscle fibers and compensate loss of muscle strength and mass. The Interreg-project GYM – Generate Your Muscle aims to develop an autologous stem cell therapy as a novel treatment for muscular dystrophies. In our subproject we aim to develop a 3D-bioprinted in vitro-vessel-on-a-chip model for the investigation and improvement of mesoangioblasts homing. Biofabricated functional vessel substitutes are used for the investigation of extravasation properties of injected mesoangioblasts under dynamic conditions with higher biomimicry than conventional migration assays under static conditions. The fabrication of the models combines extrusion with drop-on-demand-printing of cell-laden hydrogels. Thereby, a perfusable channel with a confluent endothelial cell layer with or without an adjacent smooth muscle cell layer (arterioles and capillaries, respectively) embedded by an extracellular matrix containing fibroblasts is fabricated. Cytokines, such as TNF-α or SDF-1, are included to replicate and improve the underlying migration mechanism of mesoangioblasts through the endothelial cell layer. The rheological and mechanical properties of different hydrogels combinations (gelatin, fibrinogen, thrombin, collagen) are evaluated concerning their suitability for bioprinting, facilitation of cell viability, proliferation and mesoangioblasts extravasation. As the project has just started, we will present our study design and the data obtained so far.

Keywords

Muscular dystrophy; Organ-on-a-chip; Extravasation

Abstract **#503**

NEW PRINTABLE GLYCO-HYDROGELS FOR 3D-MATERIALS AND TISSUE ENGINEERING PURPOSES

Sofia Magli[P][1], Sabrina Bertini[2], Cesare Consentino[2], Luca Crippa[3], Elisa Ballarini[3], Guido Cavaletti[3], Andrew Smith[3], Fulvio Magni[3], Francesco Nicotra[1], Laura Russo[1]

[1] *Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy.*

[2] *G. Ronzoni Institute for Chemical and Biochemical Research, Milan, Italy*

[3] *Department of Medicine and Surgery, University of Milano-Bicocca, Vedano al Lambro, Italy*

Corresponding author's email: s.magli1@campus.unimib.it

In the last decades, scientific evidence has highlighted how glycans and glycan-specific binding proteins play a key role as bio-information carriers in both pathological and physiological processes. The glycosignature of ECM is particularly important to control the cell fate during these processes. Glycans are expressed in the ECM as polysaccharides in glycosaminoglycan (GAGs) and proteoglycans (PGs) and as oligosaccharides in glycoproteins. To closely mimic the ECM in 3D structures, several polysaccharides or oligosaccharides have been employed1,2; however the availability of many commercial glycans founded in human ECM are limited by the source or the synthetic effort needed for the production. The selection of polysaccharide mimetics derived from alternative sources is a valid solution for the development of ECM glyco-components. An example of polysaccharide with high biomedical potentiality is chitosan, a linear polysaccharide formed by β-1,4-linked d-glucosamine (GlcN) and N-acetyl-d-glucosamine (GlcNAc) epitopes. Chitosan is FDA approved and it has outstanding properties such as excellent biodegradability, biocompatibility, and antimicrobial activity.3 Here in this work, to mimic the ECM hybrid structure, chitosan has been employed as polysaccharidic component and conjugate with modified gelatin using click chemistry.4 The selected glycoconjugate polymers have been employed as hydrogel for 3D U87 spheroids encapsulation and 3D bioprintable material. The obtained 3D cultures were fixed and its tissue sections were studied by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) to characterize the effect of differential glycosignature and stiffness in 3D cell cultures.

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Abstract **#505**

A NOVEL 3D IN VITRO CO-CULTURE MODEL OF PRIMARY OPEN ANGLE GLAUCOMA

Hannah C Lamont$_P$[1,2], Imran Masood[1], Michael Jones[3], Alicia J El Haj[2], Liam Grover[2], Lisa J Hill[1]

[1] *Institute of Clinical Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

[2] *Healthcare Technology Institute, School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

[3] *Cell Guidance Systems Ltd, Maia Building, Babraham Bioscience Campus, Cambridge, CB22 3AT, UK*

Corresponding author's email: hcl954@student.bham.ac.uk

Primary Open Angle Glaucoma (POAG) is a leading cause of irreversible blindness globally. The main risk factor for POAG is increased intraocular pressure (IOP), due to cellular dysfunction and fibrosis in the eye's drainage structures; the Trabecular Meshwork (TM) and Schlemm's Canal (SC), leading to an inability to control fluid outflow and IOP. The mechanisms which lead to TM/SC cellular dysfunction are still not fully understood, and there is a lack of reliable TM/SC models to accurately mimic the disease pathology. The aim of this project is to design and develop a novel 3D model of the TM/SC which can accurately model POAG.

Initially, we defined the co-culture conditions for the model using transwell inserts. To achieve this, media composition and hydrogel matrix components were investigated. TM cells are embedded in varying concentrations of type 1 collagen hydrogels, defining the correct stiffness required and SC cells are cultured on the underside of insert. Basic media (DMEM, 10%FBS and 2mM of L-glutamine) is used to culture both cell types. TGFβ-2 is added to the TM/hydrogel utilising a novel PODS® technology, releasing TGFβ-2 over 3 weeks. This release profile mimics the pathological TGFβ release within the eye, as seen in POAG.

We have demonstrated that TGFβ-2 release from the PODS® led to alterations in TM cells by producing increased alpha-SMA and fibrillogenic proteins. This resulted in an increase in the overall stiffness of the TM/hydrogel. In conclusion, our novel 3D co-culture represents more accurately the TM/SC dysfunction seen in human POAG.

Keywords

Co-culture system; Fibrosis; 3D soft materials

Abstract **#506**

DESIGN AND CHARACTERIZATION OF PHOTORESPONSIVE MULTIFUNCTIONAL HYDROGEL-BASED COMPOSITE PLATFORM

Yasamin ziaiP[1], Chiara Rinoldi[1], Sylwia Pawłowska[1], Paweł Nakielski[1], Tomasz A. Kowalewski[1], Filippo Pierini[1]

[1] *Department of Biosystems and Soft Matter, Institute of Fundamental Technological Research, Polish Academy of Sciences, 02-106 Warsaw, Poland.*

Corresponding author's email: yziai@ippt.pan.pl

Skin friendly soft materials have attracted a lot of interest as sensors for biomedical signals tracking and information recording as well as antibacterial systems. In this frame, polymeric biomaterials classified as hydrogels have several features which make them perfect choices for these applications, such as high-water content, softness, biocompatibility, tunable mechanical properties, and similar physical properties to living tissues.

In this work, metal nanoparticles, i.e., silver nanocubes, were added to poly(N-isopropylacrylamide)-based hydrogel precursor in order to obtain a thermoresponsive composite system with enhanced antibacterial and electromagnetic properties. Moreover, the introduction of a poly(ethylene oxide) – poly(caprolactone) electrospun mat into the hydrogel composites creates a compatible environment for homogenous hydrogel coating, while providing mechanical and structural properties to the final system.

Systems consisted of two outer layers of composite hydrogel, and one inner layer of electrospun mat were polymerized by UV light for a short time, obtaining a compact and stable device. Chemical, morphological and optical tests were performed to investigate the structure of each layer and the properties of the whole construct, as well as the possibility to activate the platform functions by light. The synergetic effect of photothermal-responsivity, antibacterial and electromagnetic properties of the composite material was investigated, revealing that the proposed platform is a potential candidate as an antibacterial organic molecule sensor (e.g. glucose).

Keywords

hydrogel composites; stimuli-responsive nanomaterials; multifunctional sensing systems

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Abstract **#507**

CLINICAL GRADE MSC-SECRETOME PROMOTES- HUMAN CARTILAGE RECOVERY IN VITRO

Chiara Gentili[P][1], Maria Elisabetta Federica Palamà[1], Georgina Shaw[2], Simonetta Carluccio[1], Daniele Reverberi[3], Katia Cortese[1], Frank Barry[2], Mary Murphy[2]

[1] *Department of Experimental Medicine-DIMES- University of Genoa, ITALY*

[2] *Regenerative Medicine Institute (REMEDI), National University of Ireland Galway (NUI Galway), Galway, Ireland*

[3] *U.O. Molecular Pathology, IRCCS Policlinico San Martino, Genoa, Italy.*

Corresponding author's email: chiara.gentili@unige.it

Osteoarthritis (OA) is a disabling joint disorder causing articular cartilage degeneration. The use of secretome derived from bone mesenchyme stromal cells (BMSCs) represents a novel therapeutic strategy for OA. We characterized cells, secretome, extracellular vesicles (EVs), and EVs micro RNAs of hBMSCs grown in XFS compared to platelet lysate (PL), and conventional fetal bovine serum (FBS) culture system. We investigated also the therapeutic potential of conditioned media (CM) and EVs in an in vitro model of OA. We observed that XFS and PL promoted enhanced growth and viability of hBMSCs compared to the FBS medium. The secretome of these cells showed involved in homeostasis, wound healing, and angiogenesis, as well as a marked production of EVs. The biological effects of the CM and EV derived from hBMSC cultivated in XFS, and FBS-based medium was tested on IL-1α treated hACs to mimic the OA environment. We observed that under inflammatory condition hACs are able to recruit and internalize more MSC-derived EVs, especially those derived from cells cultured in our XFS system. Treatment with CM and EVs derived from XFS-cultured hBMSC inhibited IL-1α-induced expression of IL-6, IL-8, and COX-2 by hACs compared to FBS-based conditions. Moreover, hBMSC-EVs exerted a significant chondroprotective effect when pre-treatment of hACs was performed 3h before the induction of inflammation. We are currently testing the biological validity of hBMSC-EVs grown in PL medium on the same OA in vitro model. In conclusion, we develop an innovative Cell-free Extracellular Vesicle (EV)-based therapeutic approach for modulation of OA.

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Abstract **#508**

A PERFUSION BIOREACTOR CULTURE OF SILK LIVER ECM BLEND SCAFFOLDS FACILITATING FUNCTIONAL POLARITY AND MATURATION IN PRIMARY HEPATOCYTES

Guru Janani[1], Biman B. Mandal[1,2]

[1] *Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India*

[2] *Centre for Nanotechnology, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India*

Corresponding author's email: janani@iitg.ac.in

Hepatocyte polarity maintenance and functional maturation in in vitro cultured hepatocytes are primary goals to acquire major liver-specific functions. To endeavor this, the unique microenvironment with regulated pore size and mechanical stability supporting the varied cell-matrix interaction is crucial. In this study, three dimensional (3D) in vitro liver model were fabricated by blending silk fibroin and liver ECM in different ratios, and cultured with primary rat hepatocytes to accomplish the hepatocyte polarity. Physicochemical characterization like FESEM, FTIR, mechanical strength, integral stability, etc. was performed to understand the morphology and structural transition in the scaffolds. Cell proliferation and gene expression analysis of liver-specific functional markers including albumin, cytochrome P450 2E1, cytochrome P450 1A2, HNF 4α, α1 antitrypsin, SOX 9, and fibronectin revealed a significant difference in the expression pattern among variants of silk liver ECM blend scaffolds. Biochemical estimation of synthetic proteins and immunofluorescence staining analysis is in line with the gene expression analysis. Accordingly, the scaffolds are arranged in perfusion bioreactor mimicking the native liver lobule zone-specific functions, and long-term culture supported functional polarity in terms of synthetic proteins (albumin), ECM deposition (fibronectin, desmin), and bile canaliculi (cytokeratin 18). In this study, physicochemical attributes of silk liver ECM blend scaffolds established a unique pattern of functional hepatocyte polarity in each scaffold variant. Further, perfusion culture enabled maturation and functional polarity maintenance mimicking the native liver. As a whole, the in vitro model will find potential applications in bioartificial liver devices.

Keywords

Silk blend liver ECM scaffolds; Hepatocyte polarity; Bioartificial liver

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Abstract **#510**

AURICLE DECELLULARIZATION AS A POSSIBLE ORGAN REGENERATION STRATEGY

Erica Gentilin[1,2], Edi Simoni[1,2], Diego Cazzador[1,2], Mariarita Candito[1,2], Laura Astolfi[1,2]

[1] *Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Padua, Italy*

[2] *LIFELAB Programme, Consorzio per la Ricerca Sanitaria (CORIS), Veneto Region, Padua, Italy*

Corresponding author's email: erica.gentilin@unipd.it

Defects of the auricle affect physiological and psychological well-being of patients. To date, reconstruction techniques of the auricle, despite their effectiveness, are limited because of donor site morbidity, high risk of complications and surgical complexity. Innovative tissue engineering techniques based on decellularization may be reliable alternatives for treatment of pathological conditions affecting the auricle.

The aim of this study was to develop a biocompatible decellularization protocol to obtain an engineered auricle. The study involved the construction of a scaffold based on cell-free extracellular matrix to be repopulated with autologous stem cells, allowing the regeneration of the organ and avoiding the rejection reaction. Rat auricles were harvested and perfused through a bioreactor. The efficiency of decellularization was evaluated based on specific parameters. Cell loss was evaluated by hematoxylin and eosin, DAPI stains and DNA quantification. The absence of muscle fibers was evaluated by Masson's trichrome stain and the extracellular matrix preservation by Alcyan blue, van Gieson's trichrome, Weigert and PAS stains. The biocompatibility of grafts was assessed by an indirect cytotoxicity assay. The study identified an efficient decellularization protocol, able to remove epithelial, glandular and muscle tissues, leaving no visible nuclei but preserving elastic fibers, collagen and glycoproteins. The glycosaminoglycans content was slightly reduced. The protocol resulted both reproducible and biocompatible. In conclusion, a protocol allowing the decellularization of the auricle and preserving the extracellular matrix was developed in our study, representing a first step to obtain an efficient, implantable and clinically useful engineered auricle, devoid of immunogenicity.

Abstract **#511**

BIOMECHANICAL CHARACTERISATION OF HA-CAP LADEN STRONG HYDROGELS FOR BONE GRAFTING

Michael Gasik[P][1], Marina Sokolova[2], Alexandra Zühlke[1], Janis Locs[2], Dagnija Loca[2], Yevgen Bilotsky[3]

[1] *Aalto University Foundation*

[2] *Riga Technical University*

[3] *Seqvera Ltd.*

Corresponding author's email: michael.gasik@aalto.fi

Inorganic-organic composites with calcium phosphates (CaP) and biocompatible polymers have a great interest in the field of bone tissue engineering. Mechanical properties, degradation behavior and cell material interaction are the most affected characteristics resulting from the addition of inorganic materials to polymeric hydrogel networks.

In this we report developed in situ precipitation synthesis for the production of hyaluronic acid (HA)/CaP composite strong hydrogels (90 mg/mL HA) and their biomechanical properties, analyzed with a new model-free method. We have observed significant non-linear effect of CaP concentration from 0 to 60% vol. on both invariant stiffness and viscosity, as well as differences of the properties at frequencies between 0.1-20 Hz. Results indicate that a careful adjustment of the in situ precipitated CaP in these hydrogels is required to tailor biomechanical properties for chosen clinical application.

This work was partially supported by COST Action MP1306 "NEWGEN".

Abstract **#512**

MULTI-DIMENSIONAL SCAFFOLDS FOR LIVER TISSUE ENGINEERING – AN INTERDISCIPLINARY APPROACH

Rhiannon Grant[P][1,2], John Hallett[2], David Hay[2], Stuart Forbes[2], Anthony Callanan[2]

[1] *Maastricht University*

[2] *University of Edinburgh*

Corresponding author's email: r.grant@maastrichtuniversity.nl

A combination of increasing liver disease incidence and a shortage of donor organs available for patient treatment has led to a need for liver 'organoids'; lab created environments which mimic the in vivo extracellular matrix [ECM] niche and support the survival and function of hepatocytes.

A combination of biological materials supported by electrospun polymer scaffolds has demonstrated the ability to influence hepatocyte function, and with this in mind we created multidimensional scaffolds for liver tissue engineering. Several approaches were taken – decellularized human liver ECM, drug derived ECM and synthetic production of specific ECM proteins.

Scaffolds were fabricated by combining electrospun polymer matrices with each of the biofunctionalization approaches. Human hepatocytes were assessed for their response using scanning electron microscopy [SEM], mechanical and biochemical quantification, histology, and gene expression analysis. Additionally, drug derived ECM:polymer scaffolds were validated using gold standard primary human hepatocytes.

Results demonstrate that this novel approach of combining the most promising of techniques from the field of liver tissue engineering produces scaffolds capable of influencing hepatocyte function, with alterations in cell survival, albumin production and gene expression demonstrated in each multidimensional environment.

Creating multidimensional scaffolds provides a method of creating tailored, consistent environments for liver bioengineering and the investigation of cell matrix interactions and is a step on the path to providing lab grown organoids for patient transplant.

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Abstract **#513**

PRINTING OF ELECTRONICS FOR IN-VITRO BIOELECTRICAL SCAFFOLDS IN NEURAL TISSUE ENGINEERING: A FEASIBILITY STUDY

Miriam Seiti[1,2], Paola Serena Ginestra[2], Rosalba Monica Ferraro[3], Elisabetta Ceretti[2], Eleonora Ferraris[1]

[1] *Manufacturing Processes and Systems (MaPS), Mechanical Engineering, KU Leuven, Sint Katelijne Waver, Belgium*

[2] *Department of Mechanical Engineering, University of Brescia, Brescia, Italy*

[3] *Department of Molecular and Translational Medicine, "Angelo Nocivelli" Institute of Molecular Medicine, University of Brescia, Brescia, Italy*

Corresponding author's email: miriam.seiti@kuleuven.be

Neural tissue engineering (NTE) investigates the fabrication of bioengineered scaffolds, able to reproduce biomimetic microenvironments of the nervous system. Successful substrates combine morphological, electrical and biochemical cues to enhance cells guidance and, in turn, neural activity. Although a proper interface between substrates and electronics is necessary to achieve neural electrical stimulation and recording, current solutions (like microelectrode arrays) usually do not integrate electrodes with NTE substrates. In this study, we propose printing of electronics (PE) as a novel manufacturing technology to develop in-vitro NTE bioelectrical scaffolds with combined neural alignment. A feasibility study on the additive manufacturing aerosol jet-based technique is presented for in-situ printing of conductive patterns onto biomimetic scaffolds. The direct writing Aerosol Jet® Printing has proven ability in PE for electrical applications (like sensors, electrodes, interconnects,…) and microstructures production (resolutions ≥10 µm) [1],[2]. It is also able to deposit various inks (conductive, dielectric and biological solutions, viscosity range [1-100] mPas) on 2D, 3D and flexible substrates [3]. Process parameter evaluation is reported to assess the printability of a silver nanoparticle (AgNPs) ink and a poly(3,4-ethylenedioxythiophene)polystyrene sulfonate (PEDOT:PSS) ink on electrospun and micro-structured scaffolds, respectively. The printed conductive patterns show electrical resistances of R(AgNPs)~140 Ω and R(PEDOT:PSS)~280 Ω (line length~1 cm, thickness~40 layers). The printability process results more stable on the micro-structured substrates due to lower electrical dispersion and uniform surface. Furthermore, the biocompatibility tests on both substrates show a higher cell viability on PEDOT:PSS. Data concerning AgNPs toxicity indeed show a dose-dependent behaviour, according to the literature.

Keywords

neural tissue engineering; bioelectrical scaffold; printed electronics

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Abstract **#515**

GENIPIN-CROSSLINKED LOW ENDOTOXIN GELATIN MICROSPHERES FOR INTRAPERITONEAL DRUG DELIVERY AND PREVENTION OF POSTSURGICAL PERITONEAL ADHESIONS

Severien Standaert[P][1], Kaat De Clercq[1], Jos Olijve[2], Wim Ceelen[3], Chris Vervaet[1], Valérie Vanhoorne[1]

[1] *Laboratory of Pharmaceutical Technology, Ghent University, Ottergemsesteenweg 460, 9000, Ghent, Belgium*

[2] *Rousselot, Meulestedekaai 81, 9000 Ghent, Belgium*

[3] *Department of Gastro-intestinal Surgery, Ghent University Hospital, Corneel Heymanslaan 10, 9000, Ghent, Belgium*

Corresponding author's email: severien.standaert@ugent.be

Epithelial ovarian cancer is one of the leading causes of death from gynecological malignancies in the western world. It is often diagnosed at an advanced stage with peritoneal metastases present because of the non-specific nature of the presenting symptoms. To improve treatment outcome, intraperitoneal (IP) chemotherapy has been added to the standard treatment modality since IP chemotherapy can achieve higher locoregional drug concentrations, while avoiding systemic toxicity.
As peritoneal adhesions are a very common complication after surgery, it hampers the uniform drug distribution in the peritoneal cavity, thereby reducing the efficacy of intraperitoneal chemotherapy after cytoreductive surgery.

Low endotoxin spheroid gelatin microspheres (MS) were prepared and crosslinked with genipin (GP-MS). The degree of crosslinking allowed to tailor the degradation rate of GP-MS, hence their residence time. In vivo studies in mice showed excellent biocompatibility, distribution and degradation characteristics of GP-MS. It was shown that the used low endotoxin gelatin-based MS are a promising strategy to prevent postoperative peritoneal adhesions.

Next, GP-MS were loaded with paclitaxel (PTX) and were retained within the peritoneal cavity from several days up to weeks depending on their degree of crosslinking. PTX release was sustained over time with an initial burst release followed by a slow release. Further, the MS decreased viability of ovarian cancer cells in a time- and concentration dependent manner. These data suggest that low endotoxin gelatin-based MS can be a promising IP drug delivery system to prevent recurrent ovarian cancer.1
Future work focuses on optimization of the formulation by testing different crosslinkers.

Keywords

gelatin; intraperitoneal; PREVENTION OF POSTSURGICAL PERITONEAL ADHESIONS

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Abstract **#516**

BUILDING A VASCULARIZED SPHEROID PATCH FROM THE BOTTOM UP

Mariana I. Neves$_P$[1,2,3], Daniel T. O. Carvalho[1,2], Tália Feijão[1,2], Ricardo M. P. da Silva[1,2], Cristina C. Barrias[1,2,4]

[1] *I3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

[2] *INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal*

[3] *FEUP – Faculdade de Engenharia, Universidade do Porto, Porto, Portugal*

[4] *ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal*

Corresponding author's email: mariana.neves@i3s.up.pt

The bottom-up assembly of spheroids is a promising biofabrication alternative to generate functional tissues of clinically-relevant size. The use of spheroids as building blocks is often challenging due to the formation of dense structures with hypoxic regions that negatively impact cells viability and/or function, which may ultimately result in deficient scaffold-host integration and implant failure. Herein, patch-like constructs composed of optimized vascularized spheroids showing ability to generate capillary-like endothelial sprouts are described and proposed as a promising bottom-up approach for tissue engineering.

Spheroids with mesenchymal stem/stromal cells (MSC) and outgrowth endothelial cells (OEC) were generated [1]. MSC-to-OEC ratio and spheroids maturation time were tested for optimal OEC survival/organization. Spheroid-based patches were formed loading cells (1:1 ratio) into a square-shaped microwell agarose-mold and allowing them to aggregate into a single structure for 18h. Single spheroids and/or patches were analyzed for: size, metabolic activity, cell/ECM organization, sprouting potential and surface stiffness.

The 1:1 cell ratio enabled the production of spheroids with optimal OEC retention and organization into vascular-like structures, stabilized by endogenous ECM, both interstitial and basement membrane-like (at the periphery of vascular structures). Maturation time influenced spheroid sprouting ability, with 1-day-old spheroids showing extensive OEC sprouting, as opposed to matured spheroids that presented significantly inhibited sprouting. Spheroid-based patches had consistent square shape and size and exhibited similar sprouting potential to individual spheroids.

Spheroid-based patches stand out as a promising tool for building scaffold-free vascularized 3D constructs for tissue engineering in a bottom-up assembly approach.

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Abstract **#517**

ELECTRICALLY CONDUCTIVE BIODEGRADABLE SILK FIBROIN/CARBON NANOFIBER SCAFFOLDS FOR CARDIAC TISSUE ENGINEERING

Yigithan Tufan[P][1], Hayriye Öztatlı[2], Bora Garipcan[2], Batur Ercan[1,3,4]

[1] *Department of Metallurgical and Materials Engineering, Middle East Technical University, Turkey*

[2] *Institute of Biomedical Engineering, Boğaziçi University, Turkey*

[3] *Biomedical Engineering Program, Middle East Technical University, Turkey*

[4] *BIOMATEN, Center of Excellence in Biomaterials and Tissue Engineering, Middle East Technical University, Turkey*

Corresponding author's email: yigithan@metu.edu.tr

Cardiac tissue engineering applications require electrically conductive platforms to transmit electrical impulses throughout damaged region of myocardium [1]. Concurrently, cardiac patches should ensure sufficient mechanical properties to preserve their mechanical integrity under physiologically relevant stress levels [2]. Besides, a porous microstructure is essential to provide requisite surface area for cellular functions, while allowing nutrient, waste and oxygen transport with the surrounding tissues [3]. Furthermore, biodegradability of a cardiac patch is another requirement to prevent a secondary surgery [4]. To address all of the aforementioned requirements, we fabricated electrically conductive biodegradable silk fibroin (SF)/carbon nanofiber (CNF) scaffolds to be used in cardiac tissue applications. Detailed characterization of physicochemical, mechanical, electrical and structural properties of the scaffolds as well as their biodegradation characteristics were assessed. Results revealed that incorporation of CNFs into porous SF matrix led to similar mechanical and electrical properties to that of heart muscle tissue and provided a control over its degradation through altering fabrication parameters. A potential secondary bonding mechanism was proposed to explain the interactions between SF polypeptides and CNFs that resulted in enhanced scaffold properties. Moreover, degradation dependent property changes of SF/CNF nanocomposites were investigated to simulate their time dependent performance in vitro. Biological properties of SF/CNF nanocomposites were also assessed using induced pluripotent stem cells (iPSCs). Enhanced viability, metabolic activity and cardiomyogenic differentiation of iPSCs on SF/CNF nanocomposites revealed their potential for use in cardiac tissue engineering applications.

Keywords

Silk Fibroin; Carbon Nanofibers; Induced Pluripotent Stem Cells

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Abstract **#518**

EXAMINATION OF FOREIGN BODY RESPONSE AGAINST SUBCUTANEOUS IMPLANTATION BY UTILIZING RAMAN MICROSPECTROSCOPY

Chuan-En Lu[P][1], Julia Marzi[1,2,3], Daniel Alejandro Carvajal Berrio[1,2], Shannon L Layland[1], Garry Duffy[4], Katja Schenke-Layland[1,3,5]

[1] *Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany*

[2] *Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University Tübingen, Germany*

[3] *NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*

[4] *Anatomy & Regenerative Medicine Institute, School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Ireland*

[5] *Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA*

Corresponding author's email: chuan-en.lu@uni-tuebingen.de

Implantable devices have been developed for alternative treatment for various diseases such as cardiovascular disorders, skeletal diseases, chronic pain and diabetes. However, the interplay between the surface of implants and the immunological response can lead to implant failure. In this study, 2 types of synthetic polymers, Polyvinylidene fluoride (PVDF) & ChronoFlex, were tested to be potential biomaterials for future subcutaneous transplantation. A marker-independent approach via Raman Microspectroscopy was employed to monitor foreign body response (FBR) after subcutaneous implantation in regards to extracellular matrix (ECM) proteins such as collagen I and III, as well as alpha-smooth muscle actin. Principal component analysis (PCA) was performed on the Raman spectral data. Differences in the Raman bands representing amide I and III, phenylalanine, and hydroxyproline were identified, which indicated structural changes in collagen I between the two biomaterials that were not detectable by picrosirius red staining. The promising analytical approach provides a more sensitive and efficient way to characterize the ECM of the fibrotic capsule and a non-invasive tracking method to examine the progress of fibrosis.

Keywords

Raman Microspectroscopy ; foreign body response ; extracellular matrix proteins

Abstract **#519**

DEVELOPMENT OF A HYDROGEL – BASED MICROFLUIDIC MODEL OF LYMPH NODE TRANSFORMATION

Corrado Mazzaglia$_P$[1,2]

[1] *University of Cambridge*

[2] *MRC cancer unit*

Corresponding author's email: cm986@cam.ac.uk

Lymph nodes (LNs) are organs of the immune system that maintain homeostasis and trigger primary immune responses. However, in many solid tumours, LNs are also a major site of metastasis, which accounts for about 90% of cancer-related deaths [1]. LNs that are downstream of a tumour and directly drain tumour-derived factors, go through a process of transformation, which may prime the organ for metastasis, and critically, may impair the development of effective anti-tumour immune responses. We still know relatively little about the processes driving these LN changes and how they disrupt immunosurveillance from early stages of cancer. As in vivo models do not easily permit us to follow events longitudinally or to finely manipulate the environment, we set out to create a microfluidic system that incorporates in vitro and ex vivo components to model LN transformation in a controlled, tractable system. The system is designed to enable monitoring of tissue remodelling in real-time, and permits cell retrieval at the experimental end-point to detect changes at a genetic and surface marker level. In summary, this model, integrating molecular and biophysical cues in a dynamic 3D environment, allows us to more comprehensively profile the mechanisms that contribute to LN transformation, immune modulation or therapeutic responses in tumour draining lymph nodes (TDLNs) such as immunotherapy.

Keywords

Biomimetic model; Lymph node; microfluidics

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Abstract **#520**

THE THERAPEUTIC EFFECTS OF TREADMILL EXERCISE ON OSTEOARTHRITIS IN RATS BY WNT/B-CATENIN PATHWAY

Jianjing Lin[P][1], Dan Zhao[1], Ke Tao[1], Jianhao Lin[1], Qiang Liu[1]

[1] *Arthritis Clinical and Research Center, Peking University People's Hospital, Beijing, China.*

Corresponding author's email: linjianjing@bjmu.edu.cn

Our previous studies have proved that moderate-intensity treadmill exercise can alleviate OA in rats, but the underlying mechanism of exercise therapy needs further research. The Wnt/β-catenin signaling pathway can regulate the effects of mechanical stimulation on cartilage cells and healthy joints, and there is a delicate balance between the occurrence and development of OA. Based on this, we speculate that Wnt/β-catenin signaling pathway is involved in exercise therapy for OA. In order to verify this conjecture, we applied mechanical stimulation to the joints of OA modeled rats through a treadmill and injected activators BIO and inhibitors IWP-2 of the Wnt/β-catenin signaling pathway into the joint cavity of the rats to interfere with Wnt/β -catenin signaling pathway. OA development was examined using the gait test, histological and immunohistochemical analyses, micro-computed tomography (micro-CT) analysis at 4 weeks and 12 weeks after injection. We found that both the BIO and IWP-2 groups significantly improved gait and inhibited cartilage destruction and osteophyte formation at 4 weeks, but there was no significant change at 12 weeks. These investigations revealed that Wnt/β-catenin signaling pathway involved in the effect of mechanical stimulation on OA is time-dependent. Once it cannot be maintained, Wnt/β-catenin signaling pathway cannot participate in the process.

Abstract **#521**

SELF-ALIGNED FIBROUS SCAFFOLDS FOR AUTOMECHANOINDUCTION OF CELL CULTURES

Michael Gasik[P][1], Roman Ivanov[2], Jekaterina Kazantseva[3,4], Irina Hussainova[2]

[1] *Aalto University Foundation*

[2] *Tallinn University of Technology*

[3] *Center for Food and Fermentation Technologies*

[4] *CellIn Technologies LLC*

Corresponding author's email: michael.gasik@aalto.fi

Regenerative medicine and cell therapy represent a breakthrough change in paradigm in healthcare. For efficient cell adhesion, proliferation, morphogenesis and differentiation, scaffolds should properly mimic natural in vivo microenvironments and offer local conditions needed for regulation of cellular functions. Here we present a novel biocompatible scaffold for multi-dimensional cultures of cells, providing a cell culture matrix that supports cells growth in a proper fashion, capable of inducing cell morphologies resembling the true cell (tissue) morphology, preserving the metabolism, receptors activation and protein expression in a way which resembles the true situation in vivo [1].

We consider the action of these highly anisotropic nanoceramic scaffold to result in automechanoinduction of cells due to their own interaction with the substrate, without application of external forces or specific culture media, leading to changes in cells behavior, structure, functionality, and morphology. This has been demonstrated on hMSC and cancer cells reactions as well as for inflammatory markers downregulation in PMBC. Such scaffolds might present a new tool for cell studies using non-biological autostimulation.

This work was supported by Prototron Foundation (Estonia) and Estonian Research Council.

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Abstract **#522**

IN VIVO EVALUATIONS OF HUMAN CELL-ASSEMBLED EXTRACELLULAR MATRIX (CAM) YARNS.

Nicolas L'Heureux$_P$[1], Laure Magnan[1], Fabien Kawecki[1], Gaëlle Labrunie[1], Julie Izotte[2], Sébastien Marais[3], Marie-Pierre Foulc[4], Mickaël Lafoucade[5]

[1] *Univ. Bordeaux, INSERM, BIOTIS*

[2] *Univ. Bordeaux, Animal facility A2*

[3] *Univ. Bordeaux, UMS 3420 CNRS, US4 INSERM, Bordeaux Imaging Center*

[4] *Société de Recherche Rescoll*

[5] *Mickaël*

Corresponding author's email: nicolas.lheureux@inserm.fr

Cell-assembled extracellular matrix (CAM) has been used to produce vascular grafts without exogenous materials. [1-3] While these completely biological vascular grafts performed well in clinical trials, the in vivo remodeling and inflammatory response of this truly “bio” material has not yet been investigated. In this study, human CAM yarns were implanted subcutaneously in nude rats to investigate the innate immune response.

The impact of processing steps relevant to yarn manufacturing was evaluated (devitalization, decellularization, gamma sterilization, and twisting). We observed that all yarns were still present after six months, and were integrated into a non-inflamed loose connective tissue with a CAM tissue repopulated by fibroblastic cells and blood vessels. Two weeks after implantation, yarns caused minor peripheral inflammation except for gamma sterilized yarn that triggered a more intense host response dominated by M1 macrophages. Yarn mechanical strength was decreased two weeks after implantation except for the more compact “twisted” yarn. While the strength of other yarns appeared stable after initial remodeling, the gamma-sterilized yarn continued to lose mechanical strength over time and was significantly weaker than devitalized (control) yarns at six months.

This is the first study to formally demonstrate that devitalized human CAM is very long-lived in vivo and does not trigger a sustained degradative response but, rather, is very slowly remodeled. This data supports a strategy to produce human textiles from CAM yarn for regenerative medicine applications where a scaffold with low inflammation and long-term mechanical properties are critical.

Keywords

Cell-assembled extracellular matrix; Tissue-engineered yarn; Human textile

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Abstract **#523**

A LASER-STRUCTURED SURFACE WITH ANTI-INFECTIVE PROPERTIES PROMOTES OSTEOINTEGRATION AND PREVENTS INFECTION

Viviane Ständert[P][1], Kai Borcherding[2], Nicole Bormann[1], Gerhard Schmidmaier[3], Ingo Grunwald[4], Britt Wildemann[1,5]

[1] Julius Wolff Institute, BIH Center for Regenerative Therapies, Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, 13353 Berlin, Germany

[2] Department of Adhesive Bonding Technology and Surfaces, Fraunhofer Institute for Manufacturing Technology and Advanced Materials (IFAM), 28359 Bremen, Germany

[3] Center for Orthopedics, Trauma Surgery and Spinal Cord Injury, HTRG - Heidelberg Trauma Research Group, Heidelberg University Hospital, Heidelberg, Germany

[4] Industrial and Environmental Biology, Hochschule Bremen-City University of Applied Sciences, Neustadswall 30, 28199 Bremen, Germany

[5] Experimental Trauma Surgery, Department of Trauma, Hand and Reconstructive Surgery, Jena University Hospital, 07747 Jena, Germany

Corresponding author's email: Britt.Wildemann@med.uni-jena.de

Material associated infections are devastating complications in orthopedics. The “Race for the surface” with the osteointegration of the material can be influenced by surface properties and local antibiotic delivery. This study aimed at the development and in vivo testing of a porous titanium surface loaded with gentamicin.

The surface of titanium K-wires was laser structured, metal silver particles were embedded in the titanium dioxide layer, the pores were loaded with gentamicin, and the surface was mechanically tested. Biocompatibility and infection prophylaxis were assessed in rat osteointegration and infection models. K-wires were implanted in rat femura and the integration was analyzed by mechanical testing and histology. For infection prophylaxis, Staphylococcus aureus was inoculated and the bones were analyzed by histology and microbiology.

The pores revealed an amphora-like shape and the surface structuring was mechanically stable. Gentamicin loading revealed an adjustable loading capacity with a burst release within one hour. The in vivo study showed a good biocompatibility of the Porous and Genta-loaded K-wires with significantly better osteointegration compared to the CTRL. The Genta-loaded K-wires significantly reduced the bacterial colonization and the signs of osteomyelitis.

This porous surface modification shifts the “Race for the surface” in favor of the osteoblast. The mechanical stable amphora-like pores can be loaded with the required antibiotics. Due to the fast gentamicin release, the surface is protected from bacterial colonization without compromising biocompatibility and osteointegration. This approach enables for the production of “off-the-shelf” orthopedic implants with surface modification and loading with the antibiotic just prior to implantation.

Abstract **#524**

INDUCTION OF POLYANILINE AS ELECTRO-ACTIVE MATERIAL IN SILK FIBROIN LOADED NANOFIBROUS SCAFFOLD FOR TISSUE REGENERATION

Ragavi Rajasekaran[1], Preetam Guha Ray[1], Abir Dutta[1], Atul Kumar Ojha[1], Mamoni Banerjee[1], Santanu Dhara[1]

[1] *IIT Kharagpur*

Corresponding author's email: ragavirajasekaran@iitkgp.ac.in

The cross-talk between cell-matrix plays a pivotal role in tissue regeneration and off-late, conducting polymers have been inducted as alternatives for studying such interactions. However, close study reveals that incorporation of conducting polymers comprises stability of fabricated scaffolds. Herein, the study demonstrates a process to impart structural anisotropy with incorporation of Polyaniline (PANi) as conductive material, in silk fibroin (SF) loaded electrospun nanofibrous scaffold balancing scaffold stability as well as inducting opportunity to investigate cell-matrix interaction towards tissue regeneration. Blend of varying wt% of PANi was prepared with SF/Polycaprolactone (PCL) solution to electrospun stable scaffolds and SEM micrographs revealed that incorporation of PANi didn't affect fiber morphology and smooth beadfree nanofibrous scaffold with narrow fiber distribution from 150 nm to 250 nm was obtained by incorporating upto ~ .3 wt% of the conducting polymer. Further study with FTIR confirms presence of PANi in the composite scaffold while XRD demonstrated semi crystallinity. Surface analysis suggest moderately hydrophobic behavior ~ 115o, surface roughness of .075 µm, and mechanical study portrayed a young's modulus of ~ 30 MPa and nanoindentation presented an average modulus of 1.8 GPa. In vitro study with human dermal fibroblast (HDF) illustrated excellent compatibility profile of scaffold thus confirming mechanical stability of the scaffold for potential regenerative application. The successful incorporation of PANi in an optimized nanofibrous scaffold exhibited prospective opportunity to fabricate scaffold with plausible electrical conductance for regulating cell-cell and cell-matrix behavior towards enhanced tissue regeneration.

Abstract **#525**

PILLAR AND PERFUSION PLATE PLATFORMS FOR ROBUST HUMAN ORGANOID CULTURE AND ANALYSIS

Moo-Yeal Lee$_P$[1], Soo-Yeon Kang[1], Sangjoon Lee[1], Sunil Shrestha[1], Prabha Acharya[1], Pranav Joshi[1]

[1] *Cleveland State University*

Corresponding author's email: m.lee68@csuohio.edu

Human tissues derived from pluripotent stem cells ("human organoids") revolutionize in vitro disease modeling by providing multicellular architecture and function that are strikingly similar to those in vivo. This new and innovative technology, however, still lacks enough throughput and reproducibility to enable high-throughput screening (HTS) of compounds due to cumbersome organoid differentiation and maturation processes and difficulty in dispensing cells in hydrogels. Here, we overcome these challenges by developing "miniature three-dimensional (3D) bioprinting" technology and associated pillar and perfusion plate platforms for human organoid culture and analysis. High-precision, high-throughput, small-scale stem cell printing and encapsulation techniques were demonstrated on pillar plates (384PillarPlate and 36PillarPlate), which were coupled with a complementary deep well plate (384DeepWellPlate) and a perfusion well plate (36PerfusionPlate) for static and dynamic organoid culture. Bioprinted cells and aggregates in Matrigel were differentiated and matured into liver and intestine organoids on the pillar plate for in situ functional assays. The entire organoids on the pillar plate were permeabilized, fixed, stained with primary and secondary antibodies, and cleared with tissue clearing solutions simultaneously for in situ whole organoid imaging without the need for cryosectioning. The flexible pillar and perfusion well format connected by microchannels and reservoirs made it easy to change growth media for organoid culture without the use of bulky pumps and tubes. The pillar/perfusion plate platforms compatible with standard 384-well plates and HTS equipment such as automated fluorescence microscopes and microtiter plate readers could be adopted in current drug discovery efforts.

Abstract **#526**

DEVELOPMENT OF BIO-INSPIRED MELT-ELECTROWRITTEN SCAFFOLDS FOR VASCULAR REGENERATION

Angelica S. Federici[P][1,2], David A. Hoey[1,2,3], Daniel J. Kelly[1,2,3]

[1] Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

[2] Department of Mechanical, Manufacturing, & Biomedical Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland.

[3] Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland.

Corresponding author's email: federica@tcd.ie

Cardiovascular disease is one of the major causes of mortality across the world [1]. Synthetic vascular grafts (SVG) and autograft vessels are the current treatment modalities. SVGs are able to successfully substitute for large vessels but are ineffective for small diameter vessels (<6 mm) [2] due to compliance mismatch and thrombogenicity. While autografts remain the gold standard they are limited by supply and anatomical variability issues.

To address this, we utilised melt electrowriting (MEW) [3] to successfully fabricate planar MEW scaffolds with five different fibre orientations mimicking the range of collagen architectures of native vessels. Optical microscopy and scanning electron microscopy were used to investigate morphological characteristics of the fibrous scaffolds. Strips of native vessels and synthetic scaffolds were subjected to tensile testing, both in the circumferential and longitudinal directions, to compare the mechanical behaviour. Results demonstrated control over the mechanical response of the constructs resembling the typical j-shape curve of native tissue. Preliminary biological studies demonstrate good cell viability , and ECM deposition after 21 days.

After identifying the most suitable MEW substrate in terms of mechanical response, smooth muscle cells (SMC) differentiation, and ECM deposition, a tubular MEW based SVG with an anatomically inspired architecture will be used to recapitulate the vessel wall and address the issues currently associated with compliance mismatch.

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Abstract **#527**

USING SPAAC FOR THE SYNTHESIS OF VERSATILE COVALENT HYDROGELS: OUTPERFORMING THEM ALL?

Vianney Delplace$_P$[1], Nathan Lagneau[1], Boris Halgand[2], François Loll[1], Jerome Guicheux[2], Catherine Le Visage[1]

[1] *Université de Nantes, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France*

[2] *Université de Nantes, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France*

Corresponding author's email: vianney.delplace@univ-nantes.fr

Hydrogel design for cell encapsulation is a booming field of research. While many crosslinking strategies have been proposed, most of them require external stimuli or catalysts, are not entirely bioorthogonal, or have inherent limitations (e.g., limited stability, slow gelation rate). Thus, hydrogels that would be fully tunable, fast-gelling, biocompatible and, yet, easy to synthesize and use, remain to be designed. We hypothesized that using the strain-promoted azide-alkyne cycloaddition (SPAAC) as a crosslinking mechanism would allow the synthesis of versatile polysaccharide-based hydrogels, addressing all design criteria and offering new opportunities.

We first investigated the development of SPAAC hyaluronic acid (HA)-based hydrogels. HA components were easily synthesized in single-step reactions from commercially available compounds. Mixing two HA components together, we successfully obtained hydrogels under physiological conditions. Optimizing the HA molecular weight, substitution, component ratio and polymer content, we designed tunable hydrogels that form within seconds to minutes, with stiffness tunable over orders of magnitude (0.5 to 40 kPa). Shrinking, swelling or non-swelling hydrogels could be obtained. All of these HA-based gels were stable over months under physiological conditions, and yet could be degraded enzymatically within hours to days. We further showed that SPAAC crosslinking could be easily applied to other polysaccharides, as a most versatile platform for hydrogel design.

Using a model cell line, we demonstrated excellent viability (> 90%) via live/dead confocal imaging, and investigated the effect of 3D encapsulation on metabolism and proliferation. These materials are now being investigated for a breadth of applications, spanning from organoid culture to bioprinting.

Abstract **#528**

MATRIX BOUND NANOVESICLES: THE NEXT GENERATION OF ECM-BASED MATERIALS

George S. Hussey$_P$[1,2], Stephen F. Badylak[1,2]

[1] *University of Pittsburgh*

[2] *McGowan Institute for Regenerative Medicine*

Corresponding author's email: husseygs@upmc.edu

The evolutionarily conserved composition of ECM, and its critical role in the response to tissue injury, provide a compelling rationale for its use as an inductive biomaterial to promote tissue repair. However, gaps in our understanding of the compositional properties of ECM limit our ability to design and fabricate ECM biomaterials that fully capitalize upon their therapeutic potential. Although proteomic analyses have provided a detailed profile of ECM-associated proteins, the effects of incorporated (phospho)lipids and (extracellular)RNA on matrix biology, and the host tissue response, are largely unknown. Herein we show that matrix-bound nanovesicles (MBV) are an integral component of ECM bioscaffolds, and are enriched in miRNA and pro-resolving lipids with potent immunomodulatory activities. Using RNA sequencing and redox lipidomics to characterize the molecular cargo, we show that MBV are a distinct class of extracellular vesicle. We report that high levels of lysophospholipids, bioactive molecules important for wound healing, are a characteristic feature of MBV – and of the parent ECM by extension. Our results suggest that during wound healing, hydrolases release free MBV lipid mediators from their esterified into phospholipids forms, thereby augmenting their anti-inflammatory properties. The findings of the present study have significant clinical implications. Given their nanometer size, MBV can be utilized in clinical applications that would be otherwise untenable for planar ECM sheets. Delineation of the MBV immunomodulatory cargo may now allow for new perspectives on ECM-based therapeutics, especially in the design and manufacturing of biomimetic microenvironments, and the development of therapeutic strategies for advancing tissue engineering

Abstract **#529**

DYNAMICS OF VASCULARIZED DERMAL TISSUE FORMATION FROM HYDROGEL-EMBEDDED SPHEROIDS

Tália Feijão$_P$[1,2], Mariana I Neves[1,2,3], Aureliana Sousa[1,2], Ana L Torres[1,2], Ricardo M P da Silva[1,2], Cristina C Barrias[1,2,4]

[1] *I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal*

[2] *INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal*

[3] *FEUP – Faculdade de Engenharia, Universidade do Porto, Porto, Portugal*

[4] *ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal*

Corresponding author's email: mariana.neves@i3s.up.pt

Spheroids combining human dermal fibroblasts (HDF) and endothelial cells (EC) embedded in a fibrin gel may act as micro-sized centres for de novo dermal tissue formation, providing a promising injectable modular approach for skin wound healing. Herein, spheroids of HDF and endothelial progenitors (OEC, outgrowth EC) were generated under xeno-free (XF) conditions to analyse: cell organization and extracellular matrix (ECM) composition/mechanics in HDF-OEC spheroids; the effect of spheroids maturation stage on OEC sprouting potential and secretion of angiogenesis-related proteins; and the dynamics of endothelial tubulogenesis and dermal tissue formation from fibrin-embedded XF spheroid units.

Spheroids combining HDF (neonatal foreskin) and OEC (umbilical-cord blood) were generated and maintained in EGM-2MV(Lonza) or XF-medium (MCDB131 with Plastem® and growth factors)[1]. Spheroids were characterized for size, metabolic activity, cell/ECM organization, cell proliferation and secretome.

Within XF spheroids, OEC rapidly segregated, forming a surface monolayer and some central clusters, which eventually sprouted generating primitive networks at the core. While secretion of angiogenesis related proteins increased, endothelial sprouting potential decreased with maturation stage (1 vs.7 days). Upon fibrin-embedding, OEC (from day 1) rapidly sprouted into the gel, assembling into tubular-like structures. These sprouts organized into robust vessel-like networks, stabilized by endogenous basement membrane proteins, with impressive anastomosis between neighboring spheroids. Outward migrating HDF were highly proliferative, stained positively for vimentin and synthesized abundant amounts of interstitial ECM.

Altogether, these findings highlight the suitability of fibrin-embedded HDF-OEC spheroids as tissue-forming units for skin repair.

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Abstract **#530**

DEVELOPMENT OF A NON-VIRAL SIRNA-ACTIVATED SCAFFOLD DELIVERY SYSTEM TARGETING ANTI-CHONDROGENIC SIGNALING PATHWAYS IN THE PROGRESSION OF OSTEOARTHRITIS

Domhnall C Kelly[P][1,2], Tom Hodgkinson[1], James E Dixon[3], Kevin M Shakesheff[3], Caroline M Curtin[1,4,5], Caitriona M O'Driscoll[2,6], Fergal J O'Brien[1,4,5]

[1] *Tissue Engineering Research Group, RCSI, University of Medicine & Health Sciences, Dublin, Ireland*

[2] *Centre for Research in Medical Devices (CÚRAM), NUI Galway, Ireland*

[3] *School of Pharmacy, University of Nottingham, UK*

[4] *Trinity Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland*

[5] *Advanced Materials and Biomedical Engineering Research (AMBER) Centre, Dublin, Ireland*

[6] *Pharmacodelivery Group, School of Pharmacy, University College Cork, Ireland*

Corresponding author's email: domhnallkelly@rcsi.com

INTRODUCTION: In osteoarthritis (OA), increased cytokine production creates a positive feedback loop, activating “anti-chondrogenic” signaling pathways, which contribute to cartilage destruction [1, 2]. Delivery of siRNA provides potential for manipulating the OA microenvironment, creating conditions more conducive to stem cell recruitment and chondrogenic differentiation. This study describes the combining of a cell penetrating peptide (GET) [3] with a biomaterial scaffold, allowing for controlled delivery of siRNA in a therapeutic manner.

METHODS: siRNA targeting the NF-κB pathway complexed with GET was delivered to hMSCs and PCR was used to assess downstream chondrogenic mediators. GET-p65 siRNA formulations were incorporated into previously optimised porous coll-HyA scaffolds and scaffold architecture and particle retention were assessed using SEM and release assays. Assessment of 3D in vitro culturing of hMSCs on gene-activated scaffolds, transfection efficiency and activity of downstream mediators was carried out.

RESULTS: siRNA delivery (2D) prevented activation of the NF-κB pathway, attenuated downstream catabolic mediators and restored chondrogenic activity. SEM imaging and release assay highlighted successful retention of GET-siRNA nano-complexes within the scaffold architecture. hMSCs seeded on gene-activated scaffolds (3D) in the presence of inflammatory cytokines further demonstrated successful silencing and a subsequent reduction of downstream “anti-chondrogenic” mediators at Day 3 and 7.

DISCUSSION & CONCLUSION: Successful development of an advanced gene-activated scaffold delivery system allows for manipulation of the OA microenvironment through the silencing of the NF-κB pathway, corroborating previous results observed in 2D, and the creation of an environment more conducive to stem cell recruitment and beneficial for chondrogenic differentiation.

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Abstract **#531**

THE EFFECT OF ELECTRICAL FIELDS ON HACAT CELLS MIGRATION AND VIABILITY: A PROMISING PROSPECT FOR WOUND HEALING

Chao Lu[P][1], Cosima Prahm[1], Urska Cebron[1], Tatiana Uynuk-ool[2], Jonas Kolbenschlag[1]

[1] *Department of Hand-, Plastic, Reconstructive and Burn Surgery, BG Trauma Center Tübingen, Eberhard Karls University Tübingen, Germany*

[2] *Siegfried Weller Research Institute, Department of Trauma and Reconstructive Surgery, BG Trauma Center Tübingen, Eberhard Karls University Tübingen, Germany*

Corresponding author's email: luchaoquh@outlook.com

Introduction:

There is growing evidence that cell behavior such as migration and viability can be significantly influenced by external electric fields (EFs). Some of the behavior is thought to be closely related to wound healing.

This study aimed at investigating the effect of EFs on the migration as well as cell viability of HaCaT cells (a skin-derived cell line), as these are key steps of wound healing.

Method：

We built a set-up that was able to maintain a stable temperature and pH. EFs of 200mV/mm were introduced into the cell culture environment. Scratch-assays were performed to observe cell migration. The area of the scratches was evaluated every 12 hours for a total of 48 hours. Cell viability was evaluated by assessing mitochondrial activity using resazurin staining and total protein content was evaluated using Sulforhodamine B assay on days 1, 4, and 7.

Results:

When compared to the control group, the migration of HaCaT cells which received EFs increased significantly, after 48 hours, the EFs group had a 2.2-fold increase in migration($P < 0.001$) compared to the controls. After 7 days, the changes in cell viability, measured as the area under the curve, also showed a statistically significant difference ($p < 0.05$).

Conclusion:

The results of our study demonstrate that EFs have a positive effect on HaCaT cells migration and viability, two processes crucial for wound healing. Our findings suggest EFs can accelerate wound healing and thus could play an important role in the treatment of complex wounds.

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Abstract **#532**

THE ASSOCIATION BETWEEN ALCOHOL CONSUMPTION AND OSTEOARTHRITIS; A META-ANALYSIS OF OBSERVATIONAL STUDIES

Christopher C.H. Mak[P][1,2], Kendrick To[2], Yuhui Zhou[1], Chen Zhang[1], Stephanie Filbay[3], Wasim Khan[2]

[1] *School of Clinical Medicine, University of Cambridge*

[2] *Division of Trauma and Orthopaedics, Department of Surgery, University of Cambridge*

[3] *Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford*

Corresponding author's email: makchchris@gmail.com

Alcohol consumption is a well-known risk factor for major health conditions and can be attributed to ~3 million deaths per year worldwide[1]. Yet, there is opposing evidence in the literature for the association of alcohol consumption and osteoarthritis (OA).Three authors independently reviewed observational studies that assessed the association between alcohol use and OA. 1877 articles were assessed and 29 were selected. Cochran's Q and I2 test was calculated for heterogeneity, and fixed/random effects models were used accordingly. Meta-regression was performed via a restricted maximum likelihood approach.Of 29 included studies, 10 were cohort, 16 were cross-sectional and 3 were case-control, comprising 25192 subjects in total. Meta-analyses reported an odds ratio (OR) of association between any alcohol consumption and OA of 0.79 (0.68-0.93), suggesting a protective effect. OR of weekly consumption or more was 0.79 (0.65-0.97); no significant association was found with other patterns of consumption. Alcohol consumption was negatively associated with radiographic diagnosis (0.83 (0.70-0.98)), hand OA, knee OA, North American ethnicity and female gender. High degrees of heterogeneity was observed. During confounder analysis, grouping by unadjusted data resulted in an OR of 0.70 (0.55-0.89) but disappeared when analysing adjusted studies.Whilst weekly or more frequent alcohol consumption was negatively associated with the presence of OA, this association was abolished after confounder adjustment, due to lack of longitudinal exposure in study design, inclusion criteria for subjects and absence of confounder adjustment. Therefore, this meta-analysis provides evidence to dispel notions that alcohol use may protect against OA.

Keywords

Alcohol; Osteoarthritis; Epidemiology

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Abstract **#533**

HUMAN STEM CELL-BASED SKELETAL TISSUE ENGINEERING: BIOFABRICATION AND DEVELOPMENT OF MICROJOINT TISSUE CHIP

Rocky Tuan$_P$[1]

[1] *The Chinese University of Hong Kong*

Corresponding author's email: rt_vcoffice@cuhk.edu.hk

Degenerative joint diseases, e.g., osteoarthritis, contribute to physical disabilities. Current treatments for osteoarthritis, e.g., anti-inflammatory drugs, provide only short-term pain relief. Tissue engineering/regenerative medicine, encompassing translational application of cells/scaffolds/biological signals, is a promising approach to repair damaged/diseased tissues to restore joint function/mobility. Adult mesenchymal stem cells (MSCs), e.g., from bone marrow and adipose, with multi-lineage differentiation potential, including chondrogenesis, are a promising cartilage repair cell type. A critical component to successful cell-based cartilage tissue engineering is a biocompatible/differentiation-supportive biomaterial cell-carrier scaffold. We have recently custom-designed photocrosslinked hydrogel scaffolds capable of live cell encapsulation during fabrication, with excellent cell retention/viability/differentiation, and generated robust cartilage/bone tissue constructs. Optimized scaffolds also allow controlled delivery of chondroinductive biofactors and anti-hypertrophy agents to improve the quality of the MSC-derived engineered cartilage. Custom-designed, cell-encapsulated constructs may be formed in-situ for joint cartilage re-surfacing, potentially amenable to minimally invasive, arthroscopic procedures. We have recently applied 3D-printing and a custom-designed microbioreactor to fabricate an MSC-derived, biphasic microtissue analogue of the osteochondral junction of the articular joint. This osteochondral microphysiological system is currently being used, coupled with additional tissue constructs simulating the infrapatellar fat pad (adipose) and synovium, as a joint tissue chip (microJoint) with a synovial fluid component, to model in-vitro osteoarthritis pathogenesis, e.g., exposure to pro-inflammatory agents, and to study biological and pharmacological influences on osteochondral health. The microJoint is being used as a human cell-based platform to screen for disease-modifying osteoarthritis drugs as well as to identify the cellular/molecular targets of osteoarthritis-associated pain. [Support: NIH UG3/UH3TR002136]

Abstract **#534**

FROM MOLECULAR CONSTANTS TO MACROSCOPIC MECHANICS: TUNABLE OXIDIZED ALGINATE HYDROGELS USING MIXED DYNAMIC CROSSLINKERS

Francis LC Morgan[P][1], Matthew B Baker[1], Lorenzo Moroni[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine*

Corresponding author's email: f.morgan@maastrichtuniversity.nl

Recent advances in mechanobiology have highlighted the importance of ECM viscoelasticity in determining cellular responses to their local environment.(1) This has introduced new challenges for the rational design of hydrogels targeting specific cellular niches as both viscous (stress relaxation) and elastic (stiffness) mechanical properties must be balanced. Dynamic covalent chemistry (DCvC) is promising for this problem as the crosslinks are reversible, providing access to time-dependent viscoelastic properties. Notably, we can leverage the molecular constants associated with the reversible crosslinking reaction (Keq, k1/k-1) to modulate the stiffness and stress relaxation of a hydrogel. Here we use hydrazone and oxime crosslinks (secondary aldimines) exhibiting different molecular constants, and mixtures thereof, to tune the stiffness and stress relaxation of biocompatible oxidized alginate hydrogels. Maintaining a constant 2 wt% polymer content and total crosslinker concentration of 1 mol. equiv., we were able to tune the stiffness from 0.5 - 3.0 kPa and the stress relaxation from 10^3 s to 10^5 s by varying only the ratio of hydrazone to oxime crosslinks. While we investigated oxime and hydrazone in this work, any secondary aldimine undergoing a reversible reaction in aqueous conditions is a viable crosslinker. Indeed, varying the structure of the crosslinker changes the associated molecular constants,(2) thus giving access a wider range of mechanical properties. This broad basis for bottom-up hydrogel design is of interest for targeting viscoelastic properties of specific cellular niches, mechanobiological studies requiring fine control of mechanical properties, and as an accessible and flexible platform for designing dynamic networks.

Keywords

Dynamic covalent chemistry (DCvC); Equilibrium and rate constants; Stiffness and stress relaxation

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Abstract **#535**

INVESTIGATING THE MECHANISM FOR THE PRO-TENOGENIC EFFECT OF TENDON EXTRACELLULAR MATRIX ON HUMAN ADIPOSE-DERIVED STEM CELLS

Shuting HUANG$_P$[1], Dan WANG[1], Rocky Sung Chi TUAN[1]

[1] *Institute for Tissue Engineering and Regenerative Medicine, and School of Biomedical Sciences Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China.*

Corresponding author's email: huang.s.t@link.cuhk.edu.hk

INTRODUCTION: To address the challenges of tendon repair [1], our group previously developed a urea-based, non-enzymatic approach to prepare a soluble tendon derived ECM extract (tECM), which exhibited strong pro-tenogenic effects on human mesenchymal stem cells (hMSCs) [2]. The goal of this study is to elucidate the mechanism of the pro-tenogenic bioactivity of tECM by characterizing the active components.METHODS: tECM and human adipose-derived stem cells (hASCs) were prepared as previously described [3]. tECM fractionation was performed via either enzymatic digestion (i.e., pepsin, collagenase, hyaluronidase, chondroitinase) or size-fractionation (i.e., high-performance liquid chromatography (HPLC) in the presence of various chaotropic agents). The effects of fractionated tECM on hASC tenogenesis were assessed as previously described [2,3].RESULTS: tECM enhanced hASC proliferation and pro-tenogenic differentiation compared to fetal bovine serum (FBS) or commercial bovine collagen type I solution (Col 1, Advanced BioMatrix, USA) culture. The pro-tenogenic activity of tECM was significantly compromised by pepsin digestion, which digested tECM non-collagenous components, compared to other enzyme digestion groups. Our initial size fractionation methods via HPLC were unsatisfactory as small molecular weight tECM components (< 100 kDa) could not be separated and further technical optimization will be required.CONCLUSIONS: Our key findings include: (1) tECM is highly bioactive on hASCs, i.e., stimulating proliferation and enhancing tenogenic differentiation; and (2) non-collagenous tECM proteins are likely important active factors responsible for the tECM pro-tenogenic bioactivity [2,3]. Identification of these tECM biofactors will further support ECM-based tendon repair.ACKNOWLEDGEMENT: CUHK startup fund (TUAN) and CUHK Direct Grant (2018.092, WANG).

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Abstract **#536**

MESENCHYMAL STEM CELL THERAPY IN A MICROFLUIDIC 3D MODEL OF LUNG TUMOR SPHEROIDS EMBEDDED IN COLLAGEN

Subha Narayan Rath[P][1], Nandini Dhiman[1,2], Nadin Shagaghi[2], Mrinal Bhave[2], Huseyin Sumer[2], Peter Kingshott[2]

[1] *Indian Institute of Technology Hyderabad*

[2] *Swinburne University of Technology*

Corresponding author's email: bm15resch11002@iith.ac.in

Mesenchymal stem cells (MSC) have a distinctive property of 'homing', where they migrate to sites of injury and tumor microenvironments [1,2]. This property has brought an exciting opportunity to use them for cancer cytotherapy [3]. However, the literature reports have paradoxically shown either pro- or anti-tumor effects of MSC in vitro [3,4]. This inconsistency could be attributed to the fact that the majority of the studies were conducted on conventional 2D in vitro models that do not ideally mimic the actual tumor environment [3]. In this respect, physiologically-relevant 3D tumor-on-chip in vitro models are a viable alternative in helping obtain better clarity regarding the potential of stem cells in anticancer therapies [5]. Here, conditioned medium from human amniotic membrane mesenchymal stem cells (AMMSC-CM) or hyperthermia-treated AMMSCs (heat-AMMSC-CM) was utilized to create indirect co-culture conditions, either co-culture or heat-co-culture, respectively, for collagen-embedded lung adenocarcinoma (A549) cells in a PDMS-based microfluidic device. The A549 cells established into multicellular tumor spheroids over five days of cell culture. The AMMSC-CM resulted in significant growth and proliferation of tumor spheroids. Remarkably, heat-AMMSC-CM led to both reductions in the diameters of spheroids formed and cell proliferation. The medium containing anticancer peptide P1 was found to be the least cytotoxic to co-culture of tumor spheroids as compared to monoculture or heat-co-culture groups. AMMSCs, contrary to the previous reports claiming their tumor-inhibiting potential, exerted tumor-enhancing effect on tumor cells. This study highlighted the growing importance of developing microfluidic 3D tumor models for the testing of stem-cell-based anticancer therapies

Keywords

microfluidic 3D tumor model; mesenchymal stem cells; conditioned medium

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Abstract **#537**

FABRICATION OF HEMISPHERICAL NANOFIBROUS SCAFFOLDS FOR RETINAL TISSUE ENGINEERING BY USING A NOVEL ELECTROSPINNING APPROACH

Beatrice Belgio[1], Federica Boschetti[1], Sara Mantero[1]
[1] *LaBS, Department of Chemistry, Materials, and Material Engineering "Giulio Natta", Politecnico di Milano*
Corresponding author's email: beatrice.belgio@polimi.it

Age-related macular degeneration (AMD) affects millions of people worldwide and leads to blindness [1]. AMD is caused by age-related changes in Bruch's membrane (BM) and in the retinal pigment epithelium (RPE). Current therapies are unable to restore tissue functionality. So far, tissue engineering strategies for BM regeneration have focused on the fabrication of flat scaffolds. We propose a novel electrospinning approach to develop a hemispherical biomimetic BM substitute.
We designed a rotating collector with an innovative customizable device to fabricate hemispherical scaffolds composed of Bombyx mori silk fibroin (BMSF) and polycaprolactone (PCL). We investigated scaffold surface morphology, thickness, mechanical properties, and in vitro cytocompatibility with ARPE-19 cells.
Hemispherical scaffolds with randomly orientated nanofibers were successfully produced. They displayed a thickness of 40 µm, an average fiber diameter of 370±101 nm, a fiber packing density of 49.25±1.2%, and a Young's modulus of 13±3.6 MPa. Cells were viable after 5 days of contact with the scaffolds.
The obtained scaffolds mimic the BM native properties, including microscopic to macroscopic anatomy and mechanical properties. The BM has a random fibrillar network, a packing density of 48%, and a Young's modulus from 6 to 14 MPa [2]. Moreover, we found out that our scaffolds are biocompatible and we are carrying out additional investigation with ARPE-19 cells. However, to more closely imitate the BM, fiber diameters and scaffold thickness need to be further reduced. The successful outcome of this study will inform the treatment of an optimal substrate for RPE transplantation.

Keywords
Retina

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Abstract **#538**

THE STRATEGY TO IMPROVE THE LACK OF VASCULAR FORMATION IN CHEMICAL CROSSLINKED HYDROGEL

Shih-Yen Wei[1], Ying-Chieh Chen[1]

[1] *Department of Materials Science and Engineering, National Tsing-Hua University, Hsinchu 30013, Taiwan*

Corresponding author's email: tina84694@gmail.com

To decrease the time needed to vascularize an engineered tissue, collagen hydrogel with biodegradability, biocompatibility and permeability provided suitable microenvironments to support cell-based functional vascular networks formation. However, cells into collagen hydrogel resulted in extensive contraction and rapid degradation rate which imitated their utilization as a permanent cell-laden graft. Many studies had synthesized chemical crosslinked collagen hydrogels to decrease its volume contraction, slow down its degradation rate, and increase its mechanical stability, which unfortunately impeded the formation of blood vessels in vivo[1-3]. Here, we conjugated tyramine hydrochloride (-Ph) on collagen molecules to synthesize the chemical crosslinking collagen-Ph hydrogel crosslinked by horseradish peroxidase (HRP) and hydrogen peroxide (H2O2) as a model to understand and overcome the difficulties on engineering vascular networks. Results showed poor permeability and long-lasting inflammatory response of collagen-Ph hydrogels limited the capability of human umbilical vein endothelial (HUVECs)/mesenchymal stem cell (MSCs)-mediated vasculature formation in vivo. Thus, we added the designed spacer to increase the diffusion efficiency of the hydrogel by 2.5 times, and consumed unreacted crosslinkers to lower the density of infiltrating neutrophils and macrophages to half on day 2 to 4 after implantation. These strategies significantly increased the density of engineered cell-mediated blood vessels in collagen-Ph hydrogel by 2 times. Taken together, our data suggested that increasing diffusion of oxygen and nutrition through hydrogel and completing crosslinking reactants play important roles in engineering cell-mediating functional vascular networks formation in chemically crosslinked hydrogels.

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Abstract **#539**

HIGH-SPEED VOLUMETRIC BIOPRINTING APPROACH FOR BIOFABRICATION OF COMPLEX LIVING TISSUE STRUCTURES

Paulina Nunez Bernal[P][1], Paul Delrot[2], Damien Loterie[2], Yang Li[1], Jos Malda[1,3], Christophe Moser[2], Riccardo Levato[1,3]

[1] *Department of Orthopaedics, University Medical Center Utrecht, Utrecht University*

[2] *Laboratory of Applied Photonics Devices, École Polytechnique Fédéral Lausanne (EPFL)*

[3] *Department of Clinical Sciences, Faculty of Veterinary Science, Utrecht University*

Corresponding author's email: p.nunezbernal@umcutrecht.nl

The generation of tissue-mimetic living structures of clinically-relevant size remains a major challenge in tissue engineering. 3D-bioprinting is a promising approach to shape cell-laden biomaterials into complex architectures, but current techniques like extrusion bioprinting (EB) and digital-light processing (DLP), operating in a layer-wise fashion, pose scalability challenges and struggle to resolve certain free-form geometrical features typical of biological tissues. Herein, the concept of volumetric bioprinting (VBP) by ultra-fast visible-light tomography-inspired fabrication is introduced, demonstrating the fabrication of complex, cell-laden, cm-scale biological structures within seconds.

A gelatin-based photosensitive bioresin was developed for VBP. Centimeter-scale auricle constructs were printed in 22.7s with high volume accuracy (5.71±2.31% mismatch vs. CAD design) exposing the bioresin to visible-light (λ=405nm) shaped as multiple tomographic filtered backprojections. Printing time remained constant for samples of 1.23 and 4.14cm3, in contrast to EB (~30-90min) and DLP (~20-30min) printing. VBP-printed cells maintained high viability (>80%). Complex, mesenchymal stromal cell-laden trabecular bone models with convoluted porous networks were accurately resolved with features down to 144.69±13.55µm. After endothelial cell seeding, constructs showed enhanced neo-vessel formation. Finally, printed meniscus constructs cultured for 28-days produced fibrocartilage-like matrix and exhibited increasing compressive properties over time, approaching values comparable to native meniscal fibrocartilage (~300kPa).[1]

This study established a novel approach for bioprinting complex, tissue-like architectures within seconds. Short printing times and design freedom make this technique appealing for biomedical applications like patient-specific grafts and in vitro models. The findings shown here open new avenues for designing the next generation of biomaterial-based bioprinted constructs of clinically-relevant sizes.

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Abstract **#540**

DEVELOPMENT AND OPTIMIZATION OF SONICATION-ASSISTED METHOD ON DECELLULARIZATION OF HUMAN UMBILICAL ARTERY FOR SMALL-CALIBER VASCULAR TISSUE ENGINEERING APPLICATION

Chih-Hsun Lin[P][1,2], Kai Hsia[3], Chih-Kuan Su[1], Chien-Chin Chen[4], Hsu Ma[1], Jen-Her Lu[5]

[1] *Division of Plastic and Reconstructive Surgery, Department of Surgery, Taipei Veterans General Hospital*

[2] *Department of Surgery, School of Medicine, National Yang-Ming University*

[3] *Department of Life Science, National Taiwan University*

[4] *Department of Pathology, Ditmanson Medical Foundation Chia-Yi Christian Hospital*

[5] *Section of pediatric cardiology, Department of Pediatrics, Taipei medical university hospital*

Corresponding author's email: penul22@yahoo.com.tw

The aim of this study was to develop an effective decellularization method by ultrasound followed by wash steps. Different power value of sonication was tested for the efficacy of decellularization in human umbilical artery (sDHUA). The DNA content, histological staining, mechanical property and biocompatibility were evaluated. The sDHUA was further implanted into rat abdominal aorta. Magnetic resonance angiography (MRA) for the implanted grafts were achieved. The results demonstrated that sDHUA maintained mechanical property while preservation of collagen, elastin, fibronectin and laminin. Recellularization of HUVECs indicated that sDHUA provided niches for cells to reside in, presenting in vitro cytocompatibility . Further implantation test also indicated the fitness of sonication treated human umbilical artery as a scaffold for small-caliber tissue engieerd vascular graft.

Keywords

sonication decellularized human umbilical artery; extracellular matrix; biocompatibility

Abstract **#541**

VISCOUS FINGER AS SHORT TIME PATTERNING TECHNIQUE FOR ENGINEERING 3D THICK HYDROGELS

Min-Chun Tsai$_P$[1], Ying-Chieh Chen[1]

[1] *National Tsing Hua University Department of Materials Science and Engineering*

Corresponding author's email: j85111121@gmail.com

The size of tissues is much larger than the distance of diffusion limit, so rapidly providing blood vessels to embedded cells with sufficient nutrients and oxygen is regarded as a major strategy for the success of bioengineered tissue constructs. The engineered structure of the viscous fingers similar to natural vascular network were used as the patterning method for bioengineering vascularized tissues in our study. Viscous finger refers to a high-viscosity fluid driven by a low-viscosity fluid between parallel plates. Also, low-viscosity fluid is expected to precisely detour the microgel encapsulated in high-viscosity one and form the fingers. After crosslinking, a finger-like gel is formed between the base gel interfaces, which is a sandwich-like structure from the side view.

By controlling viscosity, flow rates of photo-crosslinkable cell-laden pre-polymer solutions, and the gap between two parallel plates, the length (0.6-1 cm), the width (100-400 μm) and the distribution density (104-9x104 # /ml) of fingers with controllable size of network structures (4-20 cm2) within less than 10 seconds could be precisely engineered. After photo-crosslinking at suitable conditions, the physical properties of base and finger gels could be manipulated individually, which successfully controlled and guided various cells spreading and growth direction to engineer vascular like structures in vitro in a short time. Besides, this technique could be applied to generate functional lumens with core-shell structure for dynamic microfluidics culture. Our 3D cell patterning technique extends the potential for modelling and fabricating thick vascularized tissues like constructs for both ex vivo and in vivo applications.

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Abstract **#542**

SYNERGISTIC CONTROL OF MECHANICS AND MICROARCHITECTURE OF 3D HYDROGEL FOR ENGINEERING HEPATIC TISSUE

Chaenyung Cha[P][1]

[1] *Ulsan National Institute of Science and Technology*

Corresponding author's email: ccha@unist.ac.kr

Culturing autologous cells with therapeutic potential derived from a patient within a bioactive scaffold to induce high functioning tissue formation is considered the ideal methodology towards realizing patient-specific regenerative medicine. Hydrogels are often employed as the scaffold material for this purpose mainly for their tunable mechanical and diffusional properties as well as presenting cell-responsive moieties. Herein, a two-fold strategy was employed to control the physicomechanical properties and microarchitecture of hydrogels to maximize the efficacy of engineered hepatic tissues. First, a hydrophilic polymeric crosslinker with a tunable degree of reactive functional groups was employed to control the mechanical properties in a wide range while minimizing the change in diffusional properties. Second, photolithography technique was utilized to introduce microchannels into hydrogels to overcome the critical diffusional limit of bulk hydrogels. Encapsulating hepatic progenitor cells derived via direct reprogramming of tissue-harvested fibroblasts, the applications of this strategy to control the mechanics, diffusion, and architecture of hydrogels in a combinatorial manner could maximize their hepatic functions. The regenerative capacity of this engineered hepatic tissue was further demonstrated using an in vivo acute liver injury model.

Keywords

Mechanics; Microarchitecture; Induced hepatic progenitor cells

Abstract **#543**

GRAFT ARCHITECTURE GUIDED SIMULTANEOUS CONTROL OF DEGRADATION AND MECHANICAL PROPERTIES OF IN SITU FORMING AND FAST DISSOLVING POLYASPARTAMIDE HYDROGELS

Chaenyung Cha[P][1]

[1] *Ulsan National Institute of Science and Technology*

Corresponding author's email: ccha@unist.ac.kr

Polyaspartamide, derived from polysuccinimide (PSI), has the advantage of conveniently presenting desired functional groups by ring-opening addition of amine-based nucleophiles to the succinimidyl ring moieties of PSI. Using diamines with varying lengths of poly(ethylene glycol) linker, polyaspartamide presenting amine groups with controllable grafting density and length, namely poly(2-hydroxyethyl aspartamide)-g-amino-poly(ethylene glycol) (PHEA-PEGAm) could be synthesized. This PHEA-PEGAm was then used to develop in situ forming hydrogels by Schiff base formation with aldehyde-containing alginate (Alg-ALD). By modulating the graft architecture (i.e. grafting length and density), the mechanical properties of resulting Alg-PHEA hydrogels could be controlled in a broad range. Remarkably, the hydrogels were shown to undergo facile degradation and complete dissolution in physiological conditions, regardless of hydrogel mechanics, by the expedited hydrolysis through the action of remaining amine groups, which was also heavily influenced by the graft architecture. Moreover, the rate of degradation could be further controlled by additional ionic crosslinking of alginate. The potential application as an injectable drug delivery system was demonstrated by measuring drug release kinetics and monitoring degradation ex vivo.

Keywords

Polyaspartamide; In situ forming; Graft architecture

Abstract **#544**

DEVELOPMENT OF FABRICATION METHODS FOR LARGE-SCALE MANUFACTURING OF CLINICAL GRADE ECM HYDROGELS.

George S. Hussey[P][1,2], Stephen F. Badylak[1,2]

[1] *University of Pittsburgh*

[2] *McGowan Institute for Regenerative Medicine*

Corresponding author's email: husseygs@upmc.edu

Extracellular matrix (ECM) hydrogels have been used in numerous preclinical and clinical applications to facilitate repair of a variety of tissues. However, a major limitation to widespread clinical translation of ECM hydrogels is the lack of large-scale manufacturing techniques. ECM hydrogel products used for preclinical and clinical studies are fabricated using laboratory-based processes that have focused on enzymatic digestion of the ECM; or the use of chaotropic extraction buffers which can affect protein structure and function. Without the development of scalable, production-ready manufacturing processes, these ECM hydrogel products will fail to meet their true potential in regenerative medicine-based clinical applications. Herein we report a method to prepare hydrogels from ECM bioscaffolds using ultrasonic cavitation. The solubilized ECM can be induced to self-assemble into a hydrogel by adjusting temperature; the material properties of the gel can be tailored by adjusting ECM concentration and sonication parameters; and the bio-inductive properties of the hydrogel appear identical to enzymatically produced hydrogels. Moreover, the ultrasonic approach lends itself to large-scale manufacturing over traditional enzymatic methods in several aspects. First, the concentration range can be expanded from 2-20mg/ml (the limit of enzymatic methods) to 25-100mg/ml using the ultrasonic cavitation method, allowing for fine-tuning of the viscoelastic properties. Second, processing time is dramatically reduced from days, to the order of minutes. ECM hydrogels prepared using ultrasonic fabrication method can conform to customizable 3D geometries and may also support the incorporation of cells or therapeutic drugs, thereby expanding their possibilities for tissue engineering and regenerative medicine-based clinical applications.

Abstract **#546**

DESIGN OF CHITOSAN MULTILAYER FIBERS CONTAINING NATURAL BIOMOLECULES FOR COMPROMISED WOUND HEALING.

Priscilla Hernandez[P][1], Jorge Delgado[1], María García[2], Argelia Rosillo[1]

[1] *Universidad de Guanajuato*

[2] *CONACyT - Universidad de Guanajuato,*

Corresponding author's email: prisii_hndz@outlook.com

When the skin is wounded, multiple cell types need to be coordinated following precise stages to bring about healing. (Rodrigues et al) These stages are coagulation, inflammation, re-epithelialization, and remodeling that are an orderly sequence of overlapping, interacting processes. (Shedoeva et al) This project aims to design a biomaterial that can be used to treat compromised wounds using hydrogels based on chitosan. Biomolecules derived from coconut, avocado and nopal are incorporated into the gel in order to match the healing stage in which they are required. Hydrogels are performed by an injection system resulting in the formation of chitosan fibers, which receive a multilayer treatment using a thickening procedure by pH changes and the elaboration of a more resistant layer using a plasma reactor. (Al Dybiat et al). As we mentioned before, the layers will release different biomolecules at each stage: In the inner layer, the avocado oil is added because it can promote collagen synthesis (Oliveira et al); required at the re-epithelialization stage. In the middle layer, nopal extract is incorporated because it can modulate the inflammatory response. (Antunes et al). Finally, in the outer layer biomolecules from coconut oil composed mainly of monolaurin are incorporated. They show antimicrobial activity due to disintegration of the lipid membrane of bacteria such as Staphylococcus aureus (Preuss et al) and thus reinforcing the antibacterial property of chitosan. As a result, we present the curves of release of the different biomolecules contained in the extracts and the structural characterization of the hydrogel.

Keywords

Chitosan ; natural biomolecules ; multilayer fibers

References

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Abstract **#547**

DECELLULARIZED HUMAN UMBILICAL ARTERIAL ARTERY MAINTAINS BLOOD FLOW, EXHIBITS ENDOTHELIALIZATION AND RECRUITS CELL BUT COULDN'T AVOID DILATATION IN XENOGENIC TRANSPLANTATION

Chih-Hsun Lin[P][1,2], Kai Hsia[3], Chih-Kuan Su[1], Chien-Chin Chen[4], Hsu Ma[1], Jen-Her Lu[5]

[1] *Division of Plastic and Reconstructive Surgery, Department of Surgery, Taipei Veterans General Hospital*

[2] *Department of Surgery, School of Medicine, National Yang-Ming University, Taipei, Taiwan*

[3] *Department of Life Science, National Taiwan University, Taipei , Taiwan*

[4] *Department of Pathology, Ditmanson Medical Foundation Chia-Yi Christian Hospital*

[5] *Section of pediatric cardiology, Department of Pediatrics, Taipei medical university hospital*

Corresponding author's email: penul22@yahoo.com.tw

We evaluated implantation of dHUAs in rat abdominal aorta as xenogenic transplantation model for up to 3 months. Totally, 12 dHUAs in length about 1-cm were implanted in rat abdominal aortas. Explantation of dHUAs was done at day-1, day-14, 1-month and 3-month for histological examination (each n=3). Magnetic resonance angiography (MRA) was also performed to evaluate graft patency at 30 & 90 days (each n=2). In histology, some red blood cells (RBCs) flew into the walls of dHUA and some inflammatory cellular (CD 68) infiltration was noted at day-1. At day 14, few endothelialization was noted in H&E, CD31, vWF stainings. There were collagen fibers, elastin fibers and α-SMA expression in vessel walls. Inflammatory cells of CD68 and CD163 were both noted. Endothelialization, collagen/elastin regeneration, α-SMA expression and inflammatory cell infiltration persisted at day-30 and day-90. MRAs demonstrated patency of dHUAs at day-30 and day-90. But graft dilatation was noted grossly and in image study. Briefly, dHUAs could maintain patency in the xenogenic model. Endothelialization was observed since day-14. Regeneration of collagen/elastin and increase of cellularity were also noted thereafter. However, dilatation of dHUAs was observed, which indicates insufficiency in transforming dHUAs into normal vascular tissues after 90-day of xenogenic transplantation.

Keywords

Decellularization; Umbilical artery; Aneurysm

Abstract **#548**

TENOPHAGES - A NOVEL TISSUE RESIDENT MACROPHAGE-LIKE CELL POPULATION IN TENDONS

Andreas Traweger[P][1], Gabriel Spitzer[1], Andrea Wagner[1], Renate Gehwolf[1], Herbert Tempfer[1], Christine Lehner[1]
[1] *Paracelsus Medical University - Institute of Tendon and Bone Regeneration*
Corresponding author's email: andreas.traweger@pmu.ac.at

Tendon disorders and injuries are one of the most common musculoskeletal disorders and recent evidence has clearly implicated the presence of immune cells and inflammatory events during early tendinopathy. However, the origin and properties of these cells remain poorly defined and the presence of tissue-resident cells fulfilling macrophage- or monocyte-related functions in healthy tendons has not been thoroughly investigated. We now describe a novel tendon-resident cell population ("tenophages") expressing the fractalkine receptor chemokine (C-X3-C motif) receptor 1 (Cx3cr1) and its highly selective ligand Cx3cl1 as well as the peptide hormone epiregulin (Ereg) in healthy tendons. Interestingly, not only did we observe expression in perivascular cells, but also very distinctly in cells embedded in the dense, collagen-rich tendon matrix fulfilling macrophage-like functions. Generally, the CX3CR1/CXCL1 signaling axis mediates chemotaxis and adhesion of immune cells and is involved in the pathogenesis and progression of various inflammatory disorders. We therefore propose that this newly identified cell population fulfils a surveillance function and is activated upon tendon tissue injury or pathological stress. Further, given the role of CX3CR1/CXCL1 in cell proliferation, angiogenesis and fibrosis upon inflammation, all hallmarks of tendinopathy, targeting this signaling axis could potentially open up new vistas in treating tendinopathies.

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Abstract **#549**

ADVANCED BIOMATERIALS FOR 3D IN VITRO CANCER MODELING

Subhas C. Kundu[P][1], Virginia Brancato[1], Banani Kundu[1], David Caballero[1], Sara Amorim[1], Catarina Abreu[1], Ricardo A Pires[1], Vitor M Correlo[1], Joaquim M Oliveira[1], Rui L Reis[1]

[1] 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, 4805-017 Barco, Guimarães, Portugal, and ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal.

Corresponding author's email: kundu@i3bs.uminho.pt

The main challenge for in vitro tumor modeling is the development of biomaterials that fully reproduce the biological, chemical and mechanical complexity of the 3D tumor microenvironment [1]. Recent advances in materials sciences and biotechnology have permitted the development of advanced biomaterials that copycat the features of the native ECM. For example, the high expression of hyaluronic acid (HA) in the tumoral ECM can be recapitulated using 3D hydrogels to study its biochemical impact in cancer cells. In particular, the binding affinity of HA with CD44 receptor can be studied, as well as the alteration of the HA mechanical properties as a function of its MW and its impact in tumor progression [2]. Similarly, silk fibroin can be exploited for tumor modeling. It can be blended with other biomaterials to negotiate its mechanical properties, biodegradability and biocompatibility. We have shown that silk fibroin, alone or in combination with gellan gum, supported the growth of cancer cells [3,4]. The co-culture of cancer cells with fibroblasts, mesenchymal or adipose stem cells mimicked better the tumor microenvironment and the heterogeneous niche regulates drug response. Next, we used collagen I as an in vivo-like model of the tumoral microenvironment to investigate the invasion capability of cancer cells. We found that cell invasiveness could be assessed by using a novel morphodynamic biomarker with high predictive power. Finally, we developed a breast cancer model to study the interaction of cancer cells and macrophages using a microfluidic chip (Acknowledgements: H2020-WIDESPREAD-2014-668983, FoReCaST) and FCT (PTDC/BTM-ORG/28168/2017; CEECIND/00352/2017; PTDC/BTM-ORG/28070/2017).

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Abstract **#550**

SILICA BACKPACKS AS A SUPPORT FOR INDIVIDUAL STEM CELLS IN TISSUE REGENERATION

Marta Maciel[1,2], Tiago Correia[2], Vítor Gaspar[2], João Rodrigues[2], Choi Insung[3], João Mano[2]

[1] CEB, University of Minho, Portugal

[2] CICECO, University of Aveiro, Portugal

[3] Department of Chemistry, KAIST, South Korea

Corresponding author's email: marta.msciel@gmail.com

Cell encapsulation strategies offer high control of cell behaviour and also protection against harsh conditions. Inspired by the biosilicification process in diatoms, tough silica shells can be engineered to protect the cells from extreme conditions. Using stem cells to reproduce such event could enhance their protection against harsh conditions, but this strategy is inadequate for long-term applications, as it restricts cell functionality and viability.1 On the other hand, partial coatings of individual mammalian cells would offer enough support while maintaining their functionality and ability for delivering molecules and other biochemical cues. Thus, we report innovative concept and strategy, where silica-based partial coatings (silica backpacks) are formed at the surface of individual stem cells. Based on electrostatic interactions and sol-gel process under mild conditions, only a part of cell surface displays a silica backpack. The results reveals that approximately 57% of cellular population present the silica backpacks and are able to survive for longer than 7 days than the cells without the backpacks in suspension conditions, forming larger aggregates, which suggests prolonged cell survival and functions in suspension. This work is an important contribution to the efficacy increase in cell-based therapies, especially in injectable cell systems. Furthermore, it also will provide a potential platform to promote cell differentiation.

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Abstract **#551**

CONTROLLED RELEASE OF BIOLOGICAL FACTORS FOR PROGENITOR CELL-MEDIATED ENDOGENOUS REPAIR OF INTERVERTEBRAL DISCS (IVDS)

Catherine Le Visage[P][1], Leslie Frapin[1], Johann Clouet[1], Claire Chedeville[1], Constantin Moraru[1], Edouard Samarut[1], Marion Fusellier[2], Jerome Guicheux[1]

[1] *Inserm U1229 RMeS Regenerative Medicine and Skeleton, Nantes, France*

[2] *ONIRIS, College of Veterinary Medicine, Nantes, France*

Corresponding author's email: catherine.levisage@inserm.fr

The recent description of progenitor cells in degenerated IVDs raised the possibility of harnessing their regenerative capacity for endogenous repair. Here, we developed an polysaccharide microbead delivery system (PMBs) for the sequential release of chemokines (CCL5) and nucleopulpogenic factors (TGF-β1 and GDF5).

PMBs (mean diameter 100 µm), obtained by a water/oil emulsification process, exhibited sustained release of biological factors for 21 days. Bioactivity of CCL5 released from PMBs on human adipose-derived stem cells (hASCs), selected to mimic disc stem/progenitors, was demonstrated using a chemotaxis assay. PMBs loaded with either chemokine exhibited an increased migratory effect on hASCs over time. The regenerative effects were investigated in ex vivo spontaneously degenerated ovine IVDs. Fluorescent hASCs were seeded on the top cartilaginous endplates (CEPs); the degenerated NPs were injected with PMBs loaded with CCL5, TGF-β1, and GDF-5. Ovine IVDs were cultured for 3, 7, and 28 days to allow for cell migration and disc regeneration. Ex vivo migration of seeded hASCs from the CEP toward the NP was evidenced, with a significantly greater distance with loaded PMBs (5.8±1.3 mm vs. 3.5±1.8 mm with no PMBs). The overall NP cellularity, collagen II and aggrecan staining intensities, and the Tie2+ progenitor cell density in the NP were increased at day 28 compared to the control groups.

Using an ovine ex vivo model of spontaneously degenerated IVDs, we have shown that PMBs loaded with CCL5/TGF-β1/GDF-5 constitute an innovative sustained delivery system for recruitment of resident progenitors, followed by their differentiation, and the subsequent regeneration of IVDs.

Abstract **#552**

ULTRA-HIGH THROUGHPUT PRODUCTION OF CELL LADEN MICROGELS FOR THE SCALABLE FABRICATION OF MICROPOROUS TISSUES

Maik Schotp[1], Malin Becker[1], Jéré van Lente[2], Tom Kamperman[1], Jeroen Leijten[1]

[1] *Department of Developmental BioEngineering, TechMed Centre, University of Twente, the Netherlands*

[2] *Department of Molecular Nanofabrication, MESA+ Institute for Nanotechnology, University of Twente, the Netherlands*

Corresponding author's email: m.r.schot@utwente.nl

INTRODUCTION: The use of microfluidic technologies to facilitate the creation of microporous annealed particle (MAP) hydrogels has garnered attention in recent years.[1] MAPs offer improved implant survival and tissue integration as compared to conventional nanoporous hydrogels.[2] However, the relatively slow production rates of microgels by current strategies has challenged translation of MAPs into clinical or industrial settings. Here, we report on the combined use of in-air microfluidics (IAMF) and Alginate-Tyramine (ATA) to enable ultra-high throughput production of dual crosslinkable microgels for the formation of MAPs.[3]

EXPERIMENTAL: ATA was produced by modifying alginate with tyramine using DMTMM-based coupling. Using IAMF, hepatic cell laden ATA microgels were prepared via ionic crosslinking under oil-free and surfactant-free conditions. ATA microgel slurries were photocrosslinked into MAPs and analyzed on viability, metabolic activity, hydraulic conductivity, micropore size distribution, pore interconnectivity, and mechanical properties.

RESULTS: UV-Vis confirmed successful ATA synthesis, which was confirmed crosslinkable via enzymatic and/or ionic crosslinking. Highly monodisperse (CV<5%) ATA microgels with diameters ranging from 50 µm to 350 µm were produced at rates as high as 3,5 mL/min. MAPs could be formed through visible light crosslinking. Confocal analysis confirmed an interconnected, porous network within MAPs. Both microgel and MAP formation associated with high viability and continued metabolic activity.

CONCLUSION: The combined use of IAMF and ATA allows for ultra-high throughput production of cell laden microgels that can be assembled into MAPs. We thus present a novel and highly scalable production platform for the creation of living microporous tissues.

Keywords

Microfluidics; Micro-Annealed Particles; Modular Tissue Engineering

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Abstract **#553**

EXPANSION OF THE EXTRAEMBRYONIC ENDODERM IN BLASTOIDS SUPPORTS POST-IMPLANTATION MORPHOGENESIS OF THE EPIBLAST

Erik J Vrij[1]

[1] *MERLN Institute for Technology-inspired Regenerative Medicine, Maastricht University, The Netherlands*

Corresponding author's email: e.vrij@maastrichtuniversity.nl

The early mammalian conceptus (blastocyst) contains two supporting extraembryonic tissues - the trophectoderm and the primitive endoderm (PrE) - that encase and guide the epiblast (Epi), which eventually forms the body. Due to the difficulty to manipulate blastocysts, the autonomous potential and the molecular interplays of PrE and Epi remain elusive. Here, we determine a combination of signaling pathways activators (Chir99021, Retinoic acid, Fgf4, and cAMP) that efficiently (80%) and rapidly (72 hours) induces naive embryonic stem cells to form a free-standing, three-dimensional PrE-/Epi-like niche in chemically-defined conditions (hydrogel-free, serum-free). Once established, this niche autonomously progresses, in minimal medium without signaling pathway modulators, into a post-implantation stage forming a pro-amniotic-like cavity surrounded by a polarized Epi covered with extraembryonic endoderm-like cells. Similarly, the expansion of the PrE-like cells within blastoids also allows for a subset of them to morphologically progress to E4.5 blastocyst-like structures containing a PrE epithelium fully enveloping the Epi compartment. Markedly, the expansion of the PrE in blastoids supports the survival, expansion and morphogenesis of a post-implantation-like Epi in vitro. Altogether, modelling early development in chemically-defined conditions delineates the sequence of pathways activities necessary to form a functional PrE/Epiblast niche, and highlights the co-development fueling post-implantation development.

Abstract **#554**

YAP DEFICIENCY IMPACTS ON GROWTH OF INDUCED PLURIPOTENT STEM CELLS ON SUB-MM SURFACE TOPOGRAPHY

Kira Zeevaert[P1], Roman Goetzke[1], Chao He[2], Ludwig Pongratz[3], Mohamed Hamdy Elsafi Mabrouk[1], Arnold Gillner[2,3], Martin Reininghaus[3], Wolfgang Wagner[1]

[1] Helmholtz Institute for Biomedical Engineering, Stem Cell Biology and Cellular Engineering, RWTH Aachen University Medical School, Aachen, Germany

[2] Chair for Laser Technology LLT, RWTH Aachen University, Aachen, Germany

[3] Fraunhofer Institute for Laser Technology ILT, Aachen, Germany

Corresponding author's email: kzeevaert@ukaachen.de

Induced pluripotent stem cells (iPSCs) can differentiate into every cell type of the human body, but little is known about how specific surface patterns impact on pluripotency or guide lineage specific differentiation. There is accumulating evidence that the Yes-associated protein (YAP), a downstream effector of the Hippo pathway, might be of particular relevance for iPSCs and their response to surface topography. In continuation of our previous work, we employed multi-beam interference technology to generate sub-µm groove-ridge structures that guide orientation and organization of iPSC colonies. Structuring of polyimide (PI) films with a UV laser with 38 ns pulse duration to generate grooves and ridges with a periodicity of 650 nm and a depth of 200 nm, led to a 6-fold elongation of iPSC colonies compared to unstructured PI. To further investigate the relevance of YAP for mechanosensing of surface patterns, we used CRISPR/Cas9 technology to generate YAP-/- iPSC knockout lines. Thereupon, we analyzed the morphology of iPSC colonies with and without YAP deficiency. The aspect ratio was significantly lower for YAP-/- iPSCs on structured PI compared to wildtype iPSCs. We currently analyze how periodic sub-µm surface topography impacts on differentiation of iPSCs, particularly towards iPSC-derived mesenchymal stromal cells. During this differentiation process the elongation and orientation of iPSCs along the structured PI decreases. Our results indicate that YAP is a key driver for mechanosensing and growth response of iPSCs to sub-µm substrates.

Abstract **#556**

ENABLING ON-DEMAND TISSUE FABRICATION USING READY-TO-USE TISSUEFABTM BIOINKS

Kevin Dicker[P][1], David Settles[1], Hanying Luo[2], Nicolynn Davis[2], Gangadhar Panambur[1]

[1] *MilliporeSigma - Polymer & Biomedical Materials R&D*

[2] *MilliporeSigma - Materials Science*

Corresponding author's email: kevin.dicker@milliporesigma.com

3D bioprinting is a revolutionary biomanufacturing tool to fabricate on-demand replacement tissue and physiologically relevant 3D tissue and disease models to accelerate drug discovery. Despite promising research advancements, challenges remain for bioprinting tissues on-demand, including the need for reproducible commercially available ready-to-use bioinks. To further the advancement of the bioprinting field, we have developed a platform of bioink formulations, TissueFabTM, that are compatible with diverse cell types and bioprinting platforms and have high batch-to-batch consistency. TissueFabTM is a family of ready-to-use bioinks based on natural and synthetic materials including Gelatin, Alginate, Hyaluronic Acid and poloxamer etc. TissueFabTM bioink formulations are designed for optimal viscosity and mechanical properties for high resolution bioprinting and to maintain high cell viability (>80%). Photoinitiator selection and concentration was optimized for post-printing curing using a range of cyto-compatible wavelengths. The printability was assessed by printing with different commercially available microextrusion based desktop bioprinters thus demonstrating the printer agnostic characteristic of the bioinks. Bioink formulations were validated for high cell viability, proliferation and metabolic activity using C2C12 mouse myoblast cells and human mesenchymal stem cells(hMSCs). Bioprinted hMSCs encapsulated in TissueFab™ - GelMA-Bone bioinks show an increase in osteogenic differentiation. Additionally, TissueFab™-GelMA-Conductive bioinks exhibit enhanced conductivity making them attractive for bioprinting electroactive tissues. TissueFabTM bioinks provide a robust platform for microextrusion-based bioprinting various cell types with both high printability and cell viability. TissueFabTM bioinks enable on-demand tissue printing which is a tangible step forward for addressing drug testing and tissue engineering challenges.

Abstract **#557**

A DEEP ANALYSIS OF MENINGEAL FIBROBLASTS FROM HUMAN BRAIN DONORS

Valentina Fantini[P][1,2], Annalisa Davin[2], Riccardo Rocco Ferrari[3], Eleonora Spampinato[3], Cecilia Pandini[3], Emanuele Tino Poloni[2], Valentina Medici[2], Orietta Pansarasa[3], Antonio Guaita[2], Cristina Cereda[3]

[1] *Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy*

[2] *Golgi-Cenci Foundation, Abbiategrasso, Italy*

[3] *Genomic and post-Genomic Center, IRCCS Mondino Foundation, Pavia, Italy*

Corresponding author's email: valentina.fantini02@universitadipavia.it

Meninges are a membrane system composed of three distinct layers, that surround the central nervous systems (CNS). During adulthood, meninges contribute to the homeostasis of the CNS, with a presence of Nestin+ cells, forming a stem cell niche. Our goal is to study in-depth the human meningeal fibroblasts (MFs) to characterize cells, and to confirm the presence of neural stem cells (NSCs), a possible source of new cortical neurons that can partially overcome the senescence and the neurodegeneration.

We isolated MFs from 6 brain donors and skin fibroblasts (SFs) as control. We evaluated the timing of cell appearance, the cell orientation, and the growing rate. We evaluated protein markers by immunofluorescence. We also performed a deep sequencing to analyze differentially expressed genes.

Fibroblast cultures from meninges and skin show a clear difference in terms of cell mobility, quicker in the MFs while SFs show a good cellular orientation respect to the MFs. Both cell lines show equal level of protein markers Fibronectin, Serpinh1, and Beta-III-tubulin, but a different Nestin expression, more evident in MFs. We found 1145 deregulated RNAs and the deregulated pathways show the involvement of focal adhesion and ECM organization, confirming the differences founded at the morphological level.

We can conclude that MFs and SFs, despite to be both fibroblasts, they are very different cells. The morphological signature, the grow rate, the transcriptional profile, and in particular the different expression of Nestin open the possibility to a new use of MFs in aging or in tissue regeneration.

Keywords

Meningeal Fibroblasts; Neuroregeneration; Aging

Abstract **#558**

THIN SILICA-BASED MICROSHEETS AS A NEW ENABLING TOOL FOR TISSUE REGENERATION

Tiago R. Correia$_P$[1], Marta M. Maciel[1,2], Sónia G. Patrício[1], João Borges[1], Pavel A. Levkin[3], João F. Mano[1]

[1] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal

[2] Centre of Biological Engineering (CEB), University of Minho, Braga, portugal

[3] Institute of Biological and Chemical Systems-Functional Molecular Systems (IBCS-FMS), Karlsruhe Institute of Technology (KIT), Germany

Corresponding author's email: trcorreia@ua.pt

The engineering of minimalistic materials with specific geometry and size have been highlighted as a promising pathway for the development of new regenerative medicine solutions. Silica, an inorganic compound commonly found in nature, arise as a cheap inorganic source and chemically flexible for this purpose. In this work, we engineered thin silica microsheets with a defined size and high-fidelity shape using superhydrophobic-superhydrophilic microarrays [1, 2] as a new enabling tool to be used as an instructive biomaterial in biomedical applications. Sol-gel technique was performed in the production of the thin silica microsheets using biomimetic superhydrophobic platforms decorated with wettable spots. The results confirmed the manufacture porous silica platforms [3] with defined design (squares and circles) and thickness around 7 µm. Biomineralization studies have been also carried out where hydroxyapatite crystals in a flower-shape form were successfully observed. Based on the promising achievements, silica platforms will be doped with bioinstructive cues to evaluate the potential of these microplatforms for guiding stem cells during differentiation process in tissue regeneration applications.

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Abstract **#559**

OPTICAL COHERENCE TOMOGRAPHY FOR THE IMAGING OF 3D CELL CULTURES – CHANCES AND OPPORTUNITIES

Enno Hachgenei[1], Robert H. Schmitt[1]

[1] *Fraunhofer Institute for Production Technology IPT*

Corresponding author's email: enno.hachgenei@ipt.fraunhofer.de

3D cell cultures are becoming more and more important in a variety of different fields of research. They are already an essential part in the testing of pharmaceuticals and in the research of disease-mechanisms, as they can mimic the real processes much better in comparison to classic 2D cell cultures.

A major limitation when working with 3D cell cultures is the limited amount of structural information that can be extracted from the 3D cell culture without destroying it. Imaging modalities like microscopy, fluorescence microscopy or light sheet microscopy all share the same limitation in penetration depth. For imaging the inner structure of the cultures it is necessary to either slice them or clear them. As 3D cultures – especially organoids – are becoming more complex and bigger structures called assembloids are being developed, it becomes yet more important to non-invasively image the cell cultures during the cultivation process.

Optical coherence tomography (OCT) is an imaging modality which generates high resolved (2-10 µm) tomographic cross sectional images while being fully non-invasive and contactless. Therefore it is possible to monitor the development of a 3D cell culture regularly and by that gain information which would otherwise be lost.

The Fraunhofer IPT is developing methods to further advance OCT for the use on 3D cell cultures, those methods include the maximization of the imaging range (normally limited to about 500 µm) and the implementation of functional extensions to the OCT-systems which allow a direct characterization of the cell behavior within the 3D cultures.

Keywords

3D cell cultures ; Optical coherence tomography ; Imaging

Abstract **#560**

BIOFABRICATION OF A TRIPHASIC VASCULO-OSSEOUS-CHONDRAL CONSTRUCT TO MODEL THE OSTEOCHONDRAL COMPLEX IN VITRO

Irene Chiesa[1,2,3], Carmelo De Maria[1,3], Anna Lapomarda[1,3], Gabriele Maria Fortunato[1,3], Paul M. Geheret[2,4], Roberto Di Gesù[2,5], Francesca Montemurro[3], Giovanni Vozzi[1,3], Riccardo Gottardi[2,4,5]

[1] *Dept. of Ingegneria dell'Informazione, University of Pisa, Pisa, Italy*

[2] *Dept. of Pediatrics, Division of Pulmonary Medicine, The Children's Hospital of Philadelphia, Philadelphia, USA*

[3] *Research Center E. Piaggio, University of Pisa, Pisa, Italy*

[4] *Dept. of Bioengineering, University of Pennsylvania, Philadelphia, USA*

[5] *Fondazione Ri.MED, Palermo, Italy*

Corresponding author's email: irene.chiesa@phd.unipi.it

The osteochondral junction is a highly organized tissue where the articular cartilage and the subchondral bone are intimately connected, representing a challenge to be reproduced in vitro [1]. In this study, we developed an in vitro vascularized osteochondral construct using a patent-pending dual-chamber bioreactor, that creates two connected but individually controlled microenvironments for cartilaginous and vasculo-osseous maturation [2]. Bone scaffolds were fabricated by 3D bioprinting gelatin, nanohydroxyapatite and genipin, and seeded with human mesenchymal stem cells (hMSCs) [3]. Cartilage scaffolds were fabricated by casting and UV crosslinking a 3:1 ratio of methacrylated gelatin (gelMA) and methacrylated hyaluronic acid (10% and 3.5% w/v, respectively, in PBS with 0.15% w/v LAP), with embedded hMSCs. Bone and cartilage scaffolds were cultured for 14 days individually in osteogenic medium (OM) and chondrogenic medium (CM), respectively [4]. Then, they were combined inside the bioreactor to form the osteochondral complex. The interconnected pore network of the bone phase was filled with human endothelial cells suspended in a hydrogel of 1:1 gelMA and fibrin [3]. A syringe pump delivered continuous media flow (2 ml/day) [2]. After 2 weeks, the constructs were removed from the bioreactor and resulted stable during handling, showing a good cohesion between phases. Live/dead assay confirmed ongoing cell vitality. Histology, immunohistochemistry and gene expression (real-time PCR) are used to validate the vascularized osteochondral model. This vasculo-osseous-chondral model represents an innovative platform to study the crosstalk between cartilage, bone, and vasculature as well as to model joint diseases and to test new drugs.

Keywords

vascularized osteochondral construct ; in vitro model ; bioreactor

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Abstract **#561**

MYOGENIC DIFFERENTIATION OF ADIPOSE STEM CELLS (ASCS) ON A 3D SILK FIBROIN SCAFFOLD

Benedikt Schäfer[P][1], Tim Ruhl[1], Gerrit Freund[1], Sabine Keutmann[2], Alicia Fernández-Colino[2], Stefan Jockenhövel[2], Justus P. Beier[1]

[1] Department of Plastic Surgery, Hand and Burn Surgery, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen

[2] Department of Biohybrid & Medical Textiles (BioTex), AME-Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany

Corresponding author's email: bschaefer@ukaachen.de

Adipose stem cells (ASCs) have multipotent differentiation capabilities, including myocytes. While no myogenesis-specific growth factors have been identified, yet, ASCs are reliably differentiated using muscle-tissue-conditioned-medium, without addition of further additives. Identification of a serum-free or human-compatible-serum is always focus for an in vivo application. We aimed to identify an optimal differentiation medium and ASCs were differentiated on a 3D-Silk-Fibroin-Scaffold with directed tubular microstructure to imitate the natural muscle environment.

Differentiation into the myogenic lineage was performed for 6 weeks using muscle-tissue-conditioned-medium. High-Glucose-Medium with 5%P/S was exposed to human muscle tissue blocks of 1cm3 for 72h. Other media with addition of Ultrosa or fetal-bovine-serum were conditioned with activated satellite-cells. We examined cellular vitality using PrestoBlue-conversion after cell seeding. Myogenic differentiation was evaluated by qRT-PCR and immunohistochemical-staining.

Successful differentiation of ASCs was achieved by conditioned medium in 2D and 3D culture. Addition of further substances, in particular of animal-origin, was prevented. Structural alignment along tailored tubes of the scaffold was evident. Muscle-tissue-conditioned-medium rendered superior effective differentiation of the ASCs into myogenic lineage than other media.

Muscle-tissue-conditioned-medium is a promising approach to myogenic ASC differentiation. Here we could show that without xenogeic supplements and without a co-culture with satellite-cells an easy and successful way to myogenic differentiation could be achieved. Identification of specific factors promoting myogenic effects observed is subject of further research. Directed growth on a 3D-Silk-Fibroin-Scaffold with directed tubular microstructure, mimicking muscle tissue, was shown. These data represent an important step towards in vivo use of animal-serum-free and biocompatible-cultured muscle tissue.

Keywords

Muscle Tissue Engineering; Adipose Stem Cells ; 3D Silk Fibroin Scaffold

Abstract **#562**

3D BIOPRINTED PECTIN-BASED COMPOSITE SCAFFOLDS FOR HMSCS DIFFERENTIATION

Anna Lapomarda$_P$[1,2], Dalila Petta[3], Aurora De Acutis[1], Maria Vittoria Colombo[3,4], Francesca Montemurro[1], Carmelo De Maria[1,2], Matteo Moretti[3,5], Giovanni Vozzi[1,2]

[1] Research Center 'E. Piaggio', University of Pisa, Largo Lucio Lazzarino 1, 56122 Pisa, Italy

[2] Department of Ingegneria dell'Informazione, University of Pisa, Via G. Caruso 16, 56122 Pisa, Italy

[3] Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale (EOC), Via Tesserete 46, 6900 Lugano, Switzerland

[4] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Via Mancinelli, 7, 20131, Milan, Italy

[5] Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, via Galeazzi, 4, 20161, Milan, Italy

Corresponding author's email: anna.lapomarda@ing.unipi.it

Pectin, a structural polysaccharide of plant cell walls, is finding increasing application in Tissue Engineering thanks to its biocompatibility, biodegradability and gelling capability [1]. However, the inadequate rheological properties limit its use in 3D bioprinting. In our previous work, we addressed this challenge by introducing (3-Glycidyloxypropyl)trimethoxysilane (GPTMS) as pectin crosslinker. The pectin-GPTMS reaction enables the bioprinting of customized three-dimensional scaffolds [2].

Here, these structures were used in combination to fibrin hydrogel to obtain composite scaffolds. We hypothesize that the stiffness of the scaffold-hydrogel system can be tuned by modifying the composition of the pectin scaffold. Therefore, three types of 3D bioprinted pectin-based scaffolds were obtained by tuning the GPTMS content and by incorporating nano-hydroxyapatite (nHA). Briefly, two water-based slurries of low-methoxyl pectin (4% w/v) and GPTMS (0.92 ml and 1.50 ml per pectin gram, respectively) were prepared [2]. An additional slurry was obtained by adding pectin (4% w/v) to a dispersion of nHA (50 % w relative to pectin) in a phosphate buffer solution (1X), and thereafter adding GPTMS (0.92 ml per pectin gram). Woodpile-shaped scaffolds were 3D bioprinted, frozen and freeze-dried.

After sterilization, the scaffolds were immersed for few seconds in a pre-gel solution of fibrin (fibrinogen 2.5 mg/ml and 0.5 U/ml thrombin) and the constructs were left to polymerize in a humidified chamber. A pool of human mesenchymal stem cells (hMSCs), isolated from four different patients, were used to assess the influence of the scaffold stiffness and composition on cell viability, proliferation and their osteogenic and chondrogenic differentiation.

Keywords

In vitro models; Pectin-based bioink; human mesenchymal stem cells

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Abstract **#563**

EVALUATION OF 3D MICROFLUIDICS BIOPRINTING PARAMETERS FOR THYROID IN VITRO MODELS

Mirco Sana$_P$[1], Lorenzp Moroni[2], Carlos Mota[2]

[1] Maastricht University, MERLN Institute of Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration department, Universiteitssingel 40, 6229ER Maastricht, the Netherlands

[2] Maastricht University, MERLN Institute of Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration department

Corresponding author's email: m.sana@maastrichtuniversity.nl

Endocrine disruptors (EDs) are an exogenous group of compounds associated with thyroid malfunctioning in the human body 1, 2. Nonetheless, there are currently no adequate in vivo or in vitro models for the preclinical testing of these compounds since both animal models and 2D cell-based are not able to closely mimic physiologic conditions 3. During the last years, 3D-bioprinting technology emerged as an innovative tool in the field of regenerative medicine and drug screening that allows the creation of 3D well-organized structures that can mirror physiologically relevant architectures 4. Nonetheless, there are still numerous challenges linked to the optimization of the bioprinting process 5.
In this work, we evaluated a new bioprinting technique based on a microfluidic system with the future objective to produce a 3D-model of the thyroid gland. Microfluidic bioprinting has different interesting features including the possibility of fine control of solutions that flow through the nozzle together with a reduced shear-stress to the cells 6. We evaluated the effect of the fundamental parameters for obtaining a fine control over the bioprinted fibers for different biomaterials. We also assessed thyroid cells behavior inside hydrogels with different compositions by observing: viability, metabolic activity and the expression of specific functional markers during the first week of culture. Finally, we are exploring different techniques in order to produce a vascularized network for the construct. Our future objective is to be able to produce more complex and physiological relevant bioprinted construct that could be relevant for next-generation in vitro cell-based assays.

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Abstract **#564**

A DYNAMIC IN VITRO MODEL OF THE HUMAN GUT MICROBIOTA

Francesco Biagini[P][1,2], Marco Calvigioni[3], Ermes Botte[1,2], Carmelo De Maria[1,2], Francesca Montemurro[1], Chiara Magliaro[1], Francesco Celandroni[3], Diletta Mazzantini[3], Emilia Ghelardi[3], Giovanni Vozzi[1,2]

[1] Research Center "E. Piaggio" - University of Pisa, Largo Lucio Lazzarino 1, 55122, Pisa, Italy

[2] Department of Information Engineering - University of Pisa, Via G. Caruso 16, 56122, Pisa, Italy

[3] Department of Translational Research and New Technologies in Medicine and Surgery - University of Pisa, Via San Zeno 37, 56127 Pisa, Italy

Corresponding author's email: francesco.biagini@phd.unipi.it

In recent years, several in vitro studies have investigated the interactions between the microorganisms that resides in the human gut microbiota and the host 1.

In this work we will study the microorganism's adhesion and the composition of a human gut microbiota taken from faecal samples cultured on an electrospun gelatine structure under dynamic conditions. Electrospun gelatine structures promote the formation of a stable biofilm compared to a positive control on a glass slide as demonstrated in previous works.

Initially we will evaluate the effects of fluid shear stress inside a culture chamber on a previously formed bacterial biofilm on the electrospun gelatine structures. To achieve this goal, we will analyse the biofilm formation using a crystal violet assay after 16 hour of flow respect to a static control. Initially E. coli and E. faecalis will be used as target bacteria for the study and then we will study the adhesion of the microorganisms from a human gut microbiota.

Finally, we will culture the human gut microbiota in dynamic condition at different times (i.e. up to 7 days). To analyse the bacterial cultures at different times, biofilm formation assays, bacterial viability assays (live/death imaging) and real time PCR and metagenomic analysis will be performed.

The realization of a dynamic in vitro model of the human gut microbiota that simulates the physiological microbial composition or an altered composition, can be used to connect this model to other cell compartments highlighting the role of the human gut microbiota on health and disease.

Keywords

Gut microbiota; Electrospinning; Shear stress

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Abstract **#566**

DEVELOPMENT OF BLOOD COMPATIBLE NANOSTRUCTURED FILMS FOR CARDIOVASCULAR PURPOSE

Beurton Jordan$_P$[1,2]

[1] *EA 3452 Cithefor University of Lorraine, France*

[2] *Inserm UMR_S 1121 Biomaterials and BioEngineering, University of Strasbourg, France*

Corresponding author's email: jordan.beurton@univ-lorraine.fr

Each year in France many patients suffer from restenosis following the implantation of a stent. These complications are due to endothelial lesions, protein adsorptions or coagulation leading to the vessel occlusion. In this context, nitric oxide (NO), an endogenous radical gasotransmitter, plays a key role1. Our aim is to design NO eluting stent coatings composed of functionalized gold nanoparticles (AuNP). Layer-by-layer polyelectrolyte films were found suitable for the creation of a surface coating. Polyallylamine and polyacrylic acid embedding AuNP were defined as the optimal construction2. Here, the key point was the blood-compatibility of these films. The formation of the initial protein layer (approximative mass of 200 to 1000 ng/cm2) on the surface is known to lead to noxious process such as hemolysis, inflammation and coagulation, thus providing antifouling to the surface is essential. Protein antifouling using graft or block PAA-b/g-Polyethylene glycol (PEG) copolymers of various length as the last layer was investigated. A graft copolymer with a high ratio of short (2k) PEG grafting provided the best protein antifouling against plasma samples with a mass of adsorbed protein of 2 ± 40 ng/cm2 (direct measurement with Quartz Cristal Microbalance). This promising result tend to demonstrate a protein resistance suitable for blood compatible surfaces. Viability of endothelial cells, proliferation assay and angiogenesis as a function of the PEG copolymer used and induced by nitric oxide release are being studied. Finally, this study aims to define the optimal design of a nitric oxide releasing blood-compatible surface coating appropriate for a cardiovascular purpose.

Keywords

Nanostructured surfaces; blood compatibility; nitric oxide release

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Abstract **#567**

A LOW-TEMPERATURE SINTERING PROCEDURE FOR THE RAPID FABRICATION OF SILK FIBROIN BIOSUBSTRATES.

Devid Maniglio[P][1,2], Alessio Bucciarelli[3], Antonella Motta[1,2], Alberto Quaranta[1], Silvia Chiera[1], Vamsi Yadavalli[4]

[1] *Department of Industrial Engineerign, University of Trento, Italy*

[2] *BIOtech Center for Biomedical Technologies, University of Trento, Italy*

[3] *Fondazione Bruno Kessler, Italy*

[4] *Department of Chemical and Life Science, Virginia Commonwealth University, Richmond, USA*

Corresponding author's email: devid.maniglio@unitn.it

Due to the unique combination of properties such as mechanical strength and toughness, biocompatibility, biodegradability, thermal stability, and easy processability [1,2] regenerated silk fibroin has been used as a functional biomaterial, adopted when a positive interaction with living tissue is required. While a plethora of micro and nanoscale architectures of silk fibroin have been explored in literature (films, fibers, microparticles, and gels), realizing large, macroscale objects of fibroin has been so far challenging. In this work we propose a low temperature sintering procedure based on a thermal-reflow to fast fabricate monoliths of solid-fibroin starting from lyophilized fibroin. Thermal-reflow is a well-known mechanism taking place when the glass transition temperature of the material is lower than the temperature used to process it. We were able to conduct a thermal reflow on lyophilized silk fibroin at 40 °C, associating water moisture absorption to a high-pressure compression in a stainless steal mold.

To optimize the amount of material undergone to the solid transition a full factorial design of experiment was used. We studied the material transformation by DSC, FTIR and SEM, proving both that the thermal-reflow occurs even a low temperature, if driven at high-pressure, and that an effective compaction can be performed only using low crystallinity fibroin. Finally preliminary in-vitro tests were conducted: human adipose-derived mesenchymal stem cells were seeded on LTS fibroin samples to evaluate cell adhesion by means of confocal microscopy. The preliminary biological results indicate the potentiality of LTS fibroin material for possible biomedical applications.

Keywords

Silk fibroin; Sintering; solid monoliths

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Abstract **#568**

DEVELOPMENT OF HYBRID COLLAGEN AND ALGINATE HYDROGEL FOR OSTEOCHONDRAL REGENERATION

Tosca Roncada$_P$[1], Anita Sanghani-Kerai[2], Gordon Blunn[1], Marta Roldo[1]

[1] *University of Portsmouth, School of Pharmacy and Biomedical Sciences, St Michael's Building, White Swan Road,Portsmouth, PO1 2DT, UK.*

[2] *Fitzpatrick Referrals Orthopaedics & Neurology Halfway Lane, Eashing Godalming, Surrey GU7 2QQ*

Corresponding author's email: tosca.roncada@port.ac.uk

Cartilage lacks the ability to self-repair when damaged and despite recent intensive research, there is still no current regenerative treatment that provides an alternative to the existing permanent joint replacement implants. Mesenchymal stem cells (MSCs) are a promising source of cells for osteochondral regeneration, however, selectively promoting MSCs differentiation into appropriate cell lineages in situ is still challenging. It has been shown that matrix stiffness is a key determinant of MSCs differentiation, suggesting that the modulation of mechanical properties of the scaffold would be important in guiding stem cell differentiation into a specific cell lineage. In this study, we developed hybrid collagen and alginate hydrogels with tunable stiffness by varying the concentration of the cross-linker. Viability and proliferation of ovine MSCs encapsulated into hydrogels of different stiffness was evaluated by calcein-AM/ethidium homodimer and carboxyfluorescein succinimidyl ester (CFSE) Celltracker staining respectively. Moreover, nutrient uptake was measured using a fluorescent glucose probe within the 3D culture. After 7 days of culture cellular morphology of oMSCs within the hydrogels was assessed using both wheat germ agglutinin (WGA) and phalloidin staining. By modifying, formulation parameters five scaffolds ranging from 3.9 kPa to 40 kPa were successfully obtained. All hydrogels assure good nutrient transport as demonstrated by glucose uptake. Further, cells were viable and proliferating within all formulations. Interestingly oMSCs retained a rounded chondrocyte morphology within all hydrogels suggesting that the hydrogel itself, without any exogenous stimuli, might have an effect on oMSCs commitment.

Abstract **#569**

PHOTODEGRADATION AND PHOTOCROSSLINKING DUEL CONTROLLED HYDROGEL

Lo-Yuan Liu$_P$[1]

[1] *The same*

Corresponding author's email: a30307a2000@gmail.com

Implantable fillers have been widely used in clinical and biomedical applications, such as skeletal muscle repair, tissue regeneration, drug release, etc. There always come out some unexpected problems following implantation, including wrong position, unexpected geometry, unsuitable stiffness and unsatisfied outcome, which need another surgery to remove implants. In the recent years, many types of hydrogel have been developed to overcome these problems, but most of them possess only one-direction to adjust their mechanical property, which is irreversible increase or decrease their stiffness. Here, we conjugated gelatin with photodegradable molecules (DBCO, NB-azide) and photocrosslinkable molecules (methacrylamide, MA) to synthesize photocrosslinking and photodegradation duel controlled gelatin-based hydrogels. We can reversibly control the mechanical properties by transdermal exposing gels to different wavelength of light. Physical properties affecting cell behavior inside hydrogels were also be investigated. Result demonstrated that this gelatin-based hydrogel is biocompatible and biodegradable. Besides, our findings also showed that dynamic controlling stiffness in situ influenced proliferation and differentiation potential of embedded human bone marrow derived mesenchymal stem cells (MSCs) in 3D culture. Moreover, we also co-cultured MSCs and human umbilical vein endothelial cell (HUVECs) into hydrogels to evaluate the effect of dynamical change in hydrogel stiffness on cell-mediated blood vessel network formation. Results demonstrated that the hydrogels could help embedded MSCs and HUVECs to grow and self-assembly form functional blood vessels in vivo . These results represented that our gels have great potential used in regenerative medicine and tissue engineering.

Abstract **#570**

ANTIMICROBIAL NANOSTRUCTURED SILVER COATINGS AND THEIR APPLICATION FOR ANTIBACTERIAL ELECTROSPUN TISSUES MANUFACTURING

Gabriela Graziani[P][1], Daniele Ghezzi[1], Giorgia Pagnotta[2], Chiara Gualandi[2], Martina Cappelletti[3], Maria Letizia Focarete[2], Michele Bianchi[4], Marco Boi[1], Nicola Baldini[1,5]

[1] *IRCCS Istituto Ortopedico Rizzoli (Rizzoli Orthopaedic Institute), Bologna, Italy*

[2] *University of Bologna, Department of Chemistry Giacomo Ciamician, Bologna, Italy*

[3] *University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy*

[4] *Istituto Italiano di Tecnologia, Ferrara, Italy*

[5] *University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy*

Corresponding author's email: gabriela.graziani2@unibo.it

Infection is among the main complications related to implantation of biomedical devices, hence its prevention is important to avoid severe complications and implants failure. To this aim, silver coating are intensively studied and largely applied to coat a variety of materials.

However, although efficacy of the films against planktonic cells is widely demonstrated, the evaluation of their effects on biofilm is largely neglected. In addition, deposition of the coatings onto porous and/or heat sensitive substrates can be challenging and frequently results into significant substrate damage, limited reproducibility, detachments, low control over film thickness and silver release, all possibly leading to cytoxocity and inflammation.

Here, antibacterial nanostructured silver-based thin films are proposed, obtained by a novel plasma-assisted technique, Ionized Jet Deposition, that permits to obtain adhesive films with a fine control over thickness. Coatings are characterized in terms of morphology and composition and their antibacterial and antibiofilm efficacy are shown against E. coli and S. aureus. Then, application to polymeric electrospun patches (Poly(L-lactic acid), Nylon, polyester polyurethane) is proposed, for manufacturing of antibacterial textiles.

Results indicate that the coatings are composed of nanosized aggregates of metallic silver, showing anti-bacterial effect against both planktonic and biofilm cells. Their application onto specific devices for testing bacterial adhesion was demonstrated to significantly reduce biofilm formation by all the bacterial strains under analysis. When deposited onto electrospun polymers, the films grew around the fibers guaranteeing anti-bacterial efficacy, without altering their shape and the porosity of the patch or causing significant damage to the substrate.

Keywords

Infection; Antibacterial Coatings; Electrospinning

Abstract **#571**

4D PRINTED SUTERELESS CLIPS FOR INTESTINAL ANASTOMOSIS EXPLOITING REGENERATED SILK AND POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE)

Carmelo De Maria[P][1,2], Irene Chiesa[1,2], Silvia Bittolo Bon[3,4], Davide Morselli[5,4], Micaela Degli Esposti[5,4], Paola Fabbri[5,4], Tommaso Viligiardi[6], Antonino Morabito[6,7], Luca Valentini[3,4]

[1] *Dept. of Ingegneria dell'Informazione, University of Pisa, Pisa, Italy*

[2] *Research Center 'E. Piaggio', University of Pisa, Pisa, Italy*

[3] *Dipartimento di Ingegneria Civile e Ambientale, Università degli Studi di Perugia, Strada di Pentima 4, 05100, Terni, Italy*

[4] *Italian Consortium for Science and Technology of Materials (INSTM), Via Giusti 9, 50121 Firenze, Italy*

[5] *Department of Civil Chemical, Environmental and Materials Engineering (DICAM), Università di Bologna, Via Terracini 28, Bologna 40131, Italy*

[6] *Department of Pediatric Surgery, Meyer Children's Hospital, Viale Pieraccini 24, 50139 Firenze, Italy*

[7] *Dipartimento Neuroscienze, Psicologia, Area del Farmaco e della Salute del Bambino NEUROFARBA, Università degli Studi di Firenze, Viale Pieraccini 6, 50121 Firenze, Italy*

Corresponding author's email: irene.chiesa@phd.unipi.it

Manual suturing is the current gold standard for intestinal anastomosis, being a difficult and time-consuming procedure [1-2]. Here, we designed and fabricated 4D printed tubes made of biobased and biocompatible regenerated silk (RS) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) to be used as sutereless clips in intestinal anastomosis. The rationale of our innovative 4D structures is the negative expansion coefficient of RS when exposed to high relative humidity (HRH), as in human body. B. mori cocoons were degummed in NaHCO3; then two solutions were prepared: i)RS/CaCl2: silk fibers dissolved in CaCl2/formic acid; ii)RS/CaCl2/KNO3: RS/CaCl2 solution with the addition of KNO3. PHBV was dissolved in chloroform [2]. Bilayer tubes were 4D printed using an extrusion-based 3D printer, with a 5mm rotating spindle. The inner layer was made of PHBV, thus creating a waterproof layer. The external layer was made of RS/CaCl2 or RS/CaCl2/KNO3. Tubes were characterized by weight variation in HRH, FTIR spectroscopy and compression test. KNO3 promotes the formation of the β-sheet intramolecular crystalline structures, stabilizing the tubes and enhancing its resistance to collapse. Finite element models were performed to analyze the contraction of the 4D printed tube under thermal stimulus. They showed that at 37°C the tubes contracts radially, compressing the intestine wall, with a bigger compression when RS/CaCl2/KNO3 is used. This result agrees with experimental evaluation of bursting pressure. In conclusion, RS/CaCl2/KNO3 -PHBV tubes are promising devices for biobased sutureless anastomosis clips. In the next future, our 4D printed device will be used in mices to verify anastomosis capabilities in vivo.

Keywords

sutureless device; intestinal anastomosis ; finite element modelling

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Abstract **#572**

DEVELOPMENT OF SEQUENTIAL CROSSLINKING HYDROGELS WITH CONTROLLABLE DRUG-RELEASING PROPERTIES FOR PREVENTION OF POST-OPERATIVE ADHESION

Hui-wen Peng[P][1], Ying-Chieh Chen[1]

[1] *Department of Materials Science and Engineering, National Tsing-Hua University, Hsinchu 30013, Taiwan, R. O. C..*

Corresponding author's email: winnie880929@gmail.com

Abdominal adhesions are common complications after medical surgery. Adept®, Interceed® and Seprafilm served as adhesion barriers are currently used in surgeries, but its drawbacks are uncontrollable biodegradability and insufficient coverage on wound with complex geometry shape, so scientists have paid more attention to develop materials acting as physical barriers to prevent postoperative adhesion. Serum albumin is abundant protein in blood, and its non-immunogenicity, low surface interaction energy, capability to transport drugs and long circulatory half-life make it suitable for being a candidate material to prepare bovine serum albumin (BSA) hydrogels. Here, tyramine (-tyr) and 2-Aminoethyl methacrylate hydrochloride (-AEMA) were single or double introduced to BSA to form BSA-tyr, BSA-AEMA and BSA-tyr-AEMA conjugates. Through chemical-crosslinking, photo-crosslinking or sequential chemical- and then photo-crosslinking, gelation time, microstructure and mechanical properties of BSA hydrogel can be modulated in situ by using different concentrations of H2O2 or UV exposure time, which depending on the severity and location of injury. Our findings demonstrated that dynamically tailoring physical properties of salicylic acid-loaded BSA hydrogels successfully controls releasing profiles of salicylic acid ex vivo. Additionally, a mouse model of serious sidewall defect-bowel abrasion was employed, and a significant reduction of post-operative peritoneal adhesion has been observed on drug-loaded hydrogel. This hydrogel-based drug delivery system is potentially promising for adhesion prevention in clinical therapy.

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Abstract **#574**

ALL-AQUEOUS INTERFACIAL POLYELECTROLYTE COMPLEXATION FOR LONG-TERM ENCAPSULATION AND CULTURE OF ADHERENT CELLS

Sara Vilabril[1], Sara Nadine[1], Catarina Neves[1], Clara Correia[1], Mara Freire[1], João Coutinho[1], Mariana Oliveira[1], João Mano[1]
[1] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193, Aveiro, Portugal*
Corresponding author's email: sara.vilabril@ua.pt

Compartmentalized systems allow the confinement of important biomolecules and cells, creating an appropriate and controlled atmosphere for bioassays, cell mimicry of regulatory mechanisms, and (micro)niches studies1. Liquid-core capsules have been suggested as semi-permeable moieties with spatial homogeneity, however, most fabrication methodologies either rely on the use of organic solvents or are time-consuming. Thus, aqueous two-phase systems emerged as a biocompatible and adaptable platform for the production of capsules for autonomous production of micro-tissues, which may be used individually or combined as a tissue engineering bottom-up strategy. Moreover, the lack of cell-adhesive sites in liquid-core structures often hampers their use as platforms for stem cell-based technologies that depend on long-term survival and multilineage differentiation. Hence, these constructs are often used to take advantage of biomaterial-free cell-directed self-organization, particularly explored for short-term cellular aggregation and consequent organoid formation.
Here, we describe the cell culture of mesenchymal stem/stromal cells and microcarriers in one-step rapidly formed robust polymeric capsules formed by interfacial complexation of oppositely charged polyelectrolytes in an all-aqueous environment. MSCs isolated from the umbilical cord were encapsulated with and without microcarriers to serve as adhesion sites. Cells were characterized for their proliferation for more than 21 days in capsules prepared either manually, by drop-wise addition (millimetric size range), or by scalable electrohydrodynamic atomization (micrometric size range). The system was cultured under static or stirred environment, showcasing its robustness, stability, and versatility. Changes in cell stemness or lineage induction, overtime, are currently being investigated, as well as the resulting product of secreted factors.

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Abstract **#575**

TECHNO-ECONOMIC ANALYSIS OF THE AUTOMATED PRODUCTION OF INDUCED PLU-RIPOTENT STEM CELLS

Bastian Nießing[P][1], Raphael Kiesel[1], Robert H. Schmitt[1]

[1] *Fraunhofer IPT*

Corresponding author's email: bastian.niessing@ipt.fraunhofer.de

Induced pluripotent stem cells (iPSC) open up the unique perspective of manufacturing cell products for drug development and regenerative medicine in tissue, disease and patient-specific forms. iPSC can be multiplied almost without restriction and differentiated into cell types of all organs. The basic requirement for clinical use of iPSC is a high number of cells, which must be produced cost-effective while maintaining reproducibility and high quality. Compared to manual cell production, its automa-tion offers a unique chance of reliable reproducibility of cells in addition to cost reduction and in-creased throughput. StemCellFactory is a prototype for a fully automated production of iPSC. How-ever, in addition to the already tested functionality of the system, it must be shown that this automa-tion brings necessary economic advantages.

This presentation shows that fully automated stem cell production offers economic advantages in addition to increased throughput. First, biological and technological basics for a fully automated pro-duction of iPSC are presented. Second, the basics for profitability calculation are presented. Third, profitability of both manual and automated production are calculated. Finally, different scenarios effecting the profitability of manual and automated production are compared

Keywords

Economic Analysis; iPSC

Abstract **#576**

TENDON EXTRACELLULAR MATRIX-INCORPORATED TOUGH HYDROGEL FACILITATES TENOGENESIS OF HUMAN ADIPOSE-DERIVED STEM CELLS

Enoch Hin-lok Poon[P][1,2], Shuting Huang[1,2], Dai Fei Elmer Ker[1,2], Rocky Sung-chi Tuan[1,2], Dan Wang[1,2]
[1] *Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China*
[2] *School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China*
Corresponding author's email: enochhlpoon@link.cuhk.edu.hk

INTRODUCTION: Tendon repair is challenging due to its hypocellularity, hypovascularity and inadequate regenerative capacity [1], underscoring the importance of developing new approaches that augment tendon repair. Building on our previous development of a urea-extracted tendon ECM fraction (tECM) that promotes tenogenesis of human adipose-derived stem cells (hASCs) [2], this study aims to evaluate the tenogenic potential of an hASC/tECM-incorporated, tough hydrogel as a novel approach to enhance tendon repair.

METHODS: hASCs were isolated from the infrapatellar fat pad tissue of total knee arthroplasty patients, and bovine tECM was prepared as previously described [2]. hASCs and tECM were incorporated into an interpenetrating polymer network hydrogel consisting of poly(ethylene glycol) diacrylate (PEGDA) and sodium alginate, with or without uniaxial cyclic tensile conditioning [3]. Ensuing analyses included mechanical tests, ex vivo degradation, biocompatibility, and pro-tenogenic effects of the tECM-enriched hydrogel on hASCs.

RESULTS: hASCs encapsulated in tECM-supplemented PEGDA-alginate hydrogel (125±14.2kPa) exhibited sustained viability (Live/Dead staining), proliferation (dsDNA assay), and enhanced tenogenic differentiation (tenogenesis-associated markers via immunofluorescence and qRT-PCR) compared to hydrogel alone group. Cyclic tensile loading further facilitated hASC tenogenesis and cell alignment along the loading direction compared to unloaded tECM-hydrogel group.

DISCUSSIONS: Main findings include (1) PEGDA-alginate hydrogel is mechanically robust and biocompatible, and (2) hASCs seeded in tECM-supplemented PEGDA-alginate hydrogel exhibit increased tenogenic differentiation, further enhanced by uniaxial cyclic loading, and provide a rational basis for application of tECM-tough hydrogels in tendon repair.

Keywords
Tendon Extracellular Matrix; Tenogenic Differentiation; Tough Hydrogel

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Abstract **#577**

5D BIOPRINTING AND DIGITAL BIO-LIBRARY

Ruben Foresti[P][1], Chiara Martini[1], Stefano Rossi[1], Massimo De Filippo[1]
[1] *Medicine and Surgery Department, University of Parma, Italy*
Corresponding author's email: ruben.foresti@unipr.it

The 5D printing (1) aims the tumour regression, supporting surgery training (2) with bionic and personalised medicine (3) via synthetic biology. The fabrication of these devices requires the implementation of new type of biofactory, the industry 5.0 (4). In the society 5.0 (5) this type of production paradigm (6) uses artificial intelligence for the optimal management of process data. The study describes how it is possible to structure the human-cyber-physical space (5) through the global standard method for society 5.0, developing the digital bio-libraries (7) linked to a specific pathology, processing data for disease regression and rehabilitation therapy. We analysed the bone loss of 12 glenoid in-vivo utilising three different methods: Pico, De Filippo (8) and FMD. The first is commonly used to define the type of therapy, evaluating the circles inscribed in the right and left bidimensional surface glenoid of the same patient; the second improves the quality of results studying the complete glenoid interaction surface; the third describes the functional glenoid trajectory supporting its digitalisation. The continuous improvement is assured realising and customising internet-of-thinks mechatronic devices, exploring the four phases of the cyberspace: big-data analysis, advanced diagnostic, new generation of intelligent manufacturing and zero-failure activity. This document aims to support bioengineers, biologists and innovation managers in the development of future industry 5.0 and digital libraries related to bionic and biosynthetic design, through the use of artificial intelligences.

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Abstract #578

IMAGE-BASED QUANTITATIVE EVALUATION OF MECHANICAL STRESS EFFECT ON IPSC AUTOMATIC CULTURE

Ryuji Kato[P][1], Kei Kanie[1]

[1] *Nagoya University*

Corresponding author's email: kato-r@ps.nagoya-u.ac.jp

By the rapid advance toward the industrialization of regenerative medicine industry, there are explosive demand for more effective and more stable cell manufacturing technologies. However, even at present, the industrial-scale cell product manufacturing with good quality control is still technologically difficult. The biggest reason of such instability and inefficiency of the present cell manufacturing is that most of culture operations are relying on human skills and experiences. Therefore, although few product manufacturing can succeed, scale-up which overwhelms the key-person's capacity is hopeless.For efficient scale up, "mechanization" to automate manual operation is essential. However, the operation mechanization is also practically difficult. Because, there are no know-hows or literature who teach the manufacture "what parameters would be critical in mechanized process". Therefore, manufacturer has to struggle for long period with costly and time-consuming trial and errors for clarifying critical parameters. However, such trial and errors are rather based on expert's feeling than logical data, therefore the investigation commonly become inefficient and costly.Our group had been working with "morphology-based analysis of cells" by combining the most recent image-processing and machine learning technologies [1]. In this study, we focused to non-invasively but quantitatively analyze the effect of mechanical stress on human induced pluripotent stem cells (iPSCs). In this work, we will show various kinds of "mechanical stresses" that can affect the culturing cells during their mechanized cell culture process, and indicate our morphological profiling can quantitate such "critical process parameter" with extremely low cost only from the early images.

Keywords

iPSC; automation; morphology

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Abstract **#579**

DEVELOPMENT OF ROBUST QUALITY DISCRIMINATION METHOD FOR HUMAN ADIPOSE-DERIVED STEM CELLS BASED ON CELL MORPHOLOGY HETEROGENEITY

Yuto Takemoto[P][1], Yuta Imai[1], Kei Kanie[1], Ryuji Kato[1]

[1] *Nagoya University*

Corresponding author's email: takemoto.yuto@i.mbox.nagoya-u.ac.jp

Human Adipose-derived Stem Cells (hADSCs), which can be collected by subcutaneous liposuction, offers a great feasibility of obtaining stem cells for therapeutic use. Furthermore, high healing ability has been reported for liver diseases and damaged skin regeneration, and its application as medicinal products have been promoted. However, a quality evaluation method in cell manufacturing has not yet been established; therefore, a highly efficient, non-invasive, and quantitative evaluation system is required.

To achieve such performances, image-based morphological information is one of the most promising and expected in-process measurement index that can be introduced in facilities easily. Our group has been reported early and robust morphology-based label-free cell evaluation methods applied to bone-marrow derived mesenchymal stem cells and induced pluripotent stem cells, and has reported the effectiveness of morphological analysis to sensitively detect "irregulars" in the culture [1].

In this work, we collected over 14000 images of hADSCs' images together with its quality decay values by the continual passages. From the images, we extracted "morphological heterogeneity information" as a fingerprint feature for quality prediction.

Our data indicated that our morphological modeling and visualization of their heterogeneity efficiently contributed to sensitively detect the irregular quality decay in hADSCs only from their early images. Our results show that the qualities of hADSCs in the manufacturing process can be modeled with good accuracy by comprehensive imaging and information analysis, therefore can be applied to the daily quality monitoring for sensitive label-free detector for cellular product manufacturing.

Keywords

ADSC; morphology; quality prediction

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Abstract **#580**

CHEMO-MECHANICAL MODELING OF ALGINATE HYDROGEL IN 3D BIO-PRINTING AND DRUG DELIVERY

Aidin Hajikhani[P][1], Michele Marino[2], Jorge-Humberto Urrea-Quintero[1], Peter Wriggers[1]

[1] *Institute of Continuum Mechanics, Leibniz University of Hanover, Germany*

[2] *Department of Civil Engineering and Computational Science, University of Rome Tor Vergata, Italy*

Corresponding author's email: hajikhani@ikm.uni-hannover.de

Alginate hydrogel is a natural polymer which has been widely used as bio-ink for the production of biological scaffolds for cell growth, and drug delivery. For stabilizing the polymeric structure of the bio-ink, alginate encounter slight crosslinking by adding an ionic stimulus (cross-linker), such as Calcium(1,2). The crosslinking process (Sol-Gel) is promoted by the diffusion of the solvent and the onset of chemical reactions in the hydrogel(3,4). Hence, the structural resolution, shape fidelity and cell survival depend on properties and characteristics of the bio-ink during the process. This work aims to develop a constitutive theory and a computational model of the crosslinking-dependent swelling and shrinking mechanisms in alginate hydrogels. The crosslinking process consisting syneresis and swelling affect the pore pressure, in turn responsible for residual stresses. Constitutive laws are defined from thermodynamical principles. The coupled system includes balance of linear and angular momentum as well as mass balance for multiple species. Consequently, the developed computational framework allows to predict stress distribution inside the hydrogel as a function of internal chemical reaction and the fluid diffusion(5). Numerical approaches for multiphysics mechanisms have been developed and implemented in a finite element framework, considering the monolithic coupling of chemical transport and mechanics(6). The importance of accounting for the coupling chemo-mechanical effect between the crosslinking shrinking and the fluid-driven swelling phenomenon is highlighted, showing the effectiveness of the proposed model.

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Abstract **#581**

AN ON-CHIP PLATFORM TO STUDY TENDON REGENERATION

Francesca Giacomini[P][1], David Barata[1], Hoon Suk Rho[1], Zeinab Tahmasebi Birgani[1], Stefan Giselbrecht[1], Roman Truckenmüller[1], Pamela Habibović[1]

[1] *Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands*

Corresponding author's email: francesca.giacomini@maastrichtuniversity.nl

Tendon injuries combined with limited healing capacity of tendon tissue have resulted in a clinical challenge and an increasing need for tendon regeneration therapies. Recapitulating the tendon extracellular matrix (ECM), predominantly consisting of aligned collagen type-I fibers, presents a bottleneck for developing such therapies [1]. Biofabrication technologies were proposed for creating collagen microstructures with controlled fiber alignment [2]. Microfluidic systems have been explored to a lesser extent [3]. Here, we present a microfluidic platform with integrated micropillar arrays to create aligned collagen fibers for studying tendon regeneration. The corresponding device was fabricated using soft lithography. Micropatterns of aligned collagen were created by flowing a collagen solution through the pillar arrays followed by crosslinking. We hypothesized that the combination of the geometry of arrayed pillars and laminar flow aligns collagen fiber to the flow direction. The results confirmed this hypothesis, showing higher fiber alignment between the pillars compared to non-pillar regions. Primary rat tenocytes were cultured on the micropatterns, and analyzed for morphology and expression of tenogenic markers including the transcription factor scleraxis (SCX). Preferred adhesion and growth of tenocytes was observed on the collagen micropatterns. Cells cultured on aligned and randomly oriented fibers showed distinct elongated and spread morphologies, respectively. SCX levels of tenocytes were significantly higher on aligned fibers compared to randomly oriented ones and regions without collagen. In conclusion, we established a microfluidic-based method to create collagen micropatterns with aligned fibers that promote tenocyte adhesion and elongation, maintain phenotype, and can provide a microenvironment for tendon regeneration studies.

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Abstract **#582**

AN OPTIMIZED BIOMIMETIC NEUROTROPHIC EXTRACELLULAR MATRIX-BASED SCAFFOLD FOR SPINAL CORD INJURY REPAIR

Ian Woods$_P$[1,2], Cian O'Connor[1,2], Lisa Frugoli[1,3], Martyna Stasiewicz[1,2], Adrian Dervan[1,2], Fergal J O'Brien[1,2]

[1] *Tissue Engineering Research Group, Royal College of Surgeons in Ireland, Dublin, Ireland*

[2] *Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland*

[3] *Department of Microbiology, Polytech Marseille, Marseille, Ireland*

Corresponding author's email: ianwoods@rcsi.com

Despite the occurrence of up to 200 cases of traumatic spinal cord injury (SCI) per million people per year in Europe, no effective treatment to restore cord function currently exists [1]. The inhibitory nature of the injury site and poor intrinsic healing capacity of injured neurons create a challenging environment for repair. Building on our group's work on peripheral nerve repair [2], we hypothesized that a scaffold with a controlled pore architecture could be functionalized with neurotrophic matrix proteins to bioactively promote axonal growth. A neurite outgrowth assay was conducted using human and mouse neuronal cells to screen neurotrophic matrix components and a synergistic combination of collagen type-IV and fibronectin exhibited potent neurotrophic effects similar to BDNF-supplementation (Brain-derived neurotrophic factor).

Both matrix proteins were combined with a hyaluronic acid (HyA) hydrogel and freeze-dried, forming a microporous scaffold containing a linear pore structure. The stiffness of the scaffolds was tuned to match the soft (<1 kPa) properties of healthy cord. Seeded neurons were examined after 3 weeks of culture using metabolic assays as well as confocal and light-sheet microscopy. The neurons exhibited excellent cellular infiltration, significantly higher ($p>0.001$) metabolic activity, increased ßIII-tubulin expression ($p>0.01$) and enhanced neurite outgrowth compared to stiffer scaffolds ($E=3.65\pm0.97$ kPa) or HyA-only controls. These data indicate that matrix components can act as potent neurotrophic signalling molecules and that scaffold stiffness plays a key role in modulating this interaction with important implications for design of bioactive scaffolds for SCI-repair. Funded by the IRFU Charitable trust and SFI-AMBER Centre.

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Abstract **#583**

IN VITRO-IN SILICO MODELS OF TUBULAR TOXIN TRANSPORT

Jasia King[P][1], Roman Truckenmüller[1], Stefan Giselbrecht[1], Aurélie Carlier[1]

[1] Maastricht University

Corresponding author's email: j.king@maastrichtuniversity.nl

2 million patients are currently suffering from end-stage renal disease worldwide, with current therapies limited to transplantation or dialysis. Dialysis is a passive system in which small unbound-toxins are removed from circulation, leading to an accumulation of protein-bound uremic toxins (PBUT). This study focuses on developing an in silico model of the PBUT transport in the proximal tubule (PT) verified by in vitro experiments. It is our intention to use the in silico model to make informed decisions on the in vitro design of a bioartificial kidney subunit.

ciPTEC-OAT1 (PT-cells expressing transporters) were seeded on collagen IV-coated porous polycarbonate membranes using 1E5cells/cm2. Uptake and kinetic transepithelial assays were performed on the monolayers with 10 µM of fluorescein as a model toxin.

A non-spatial kinetic binding model was developed using literature values for indoxyl sulphate, albumin and transporter interactions, and simulated in VCell (https://vcell.org/). The system was modelled in 2-compartments representing the basolateral and apical compartments of the in vitro PT transport. Initial conditions were set using respective values from in vitro experiments. The in vitro data was used to estimate parameters of toxin binding to the transporter and transporter density using VCell.

The fluorescein versus time curves obtained from the transport assays were fitted in the VCell model resulting in forward rates that compare well to the trends in literature. The calibrated model will be used in future work to explore the effects of altering physical and chemical parameters of the system in order to predict the optimal device design.

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Abstract **#584**

MICROWELL-BASED CULTURE OF INTESTINAL ORGANOIDS

Panagiota Kakni[1], Roman Truckenmüller[1], Pamela Habibović[1], Stefan Giselbrecht[1]

[1] Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

Corresponding author's email: panagiota.kakni@maastrichtuniversity.nl

Introduction: Organoids mimicking the development, physiology and disease of the gastrointestinal system have constituted a topic of broad interest. Here, we describe a method to culture intestinal organoids inside microwell arrays[1]. By adapting previous protocols[2,3], we were able to grow organoids for up to 13 days without embedding them in solid Matrigel but instead supplementing the medium with highly diluted Matrigel.

Methods: Microwell arrays were fabricated from thin polycarbonate films by microthermoforming[1]. Mouse intestinal organoids were preserved as previously described[2]. Following passaging, organoid fragments were resuspended in medium supplemented with 5% Matrigel, and seeded into microwell arrays.

Results: Microwells were dimensionally characterized by 3D laser scanning microscopy. The inner diameter and depth of the microwells were 500µm and 300µm, respectively. Structural analysis of organoids showed similar morphology (crypt-villus architecture surrounding a central lumen) and size as organoids grown embedded in Matrigel. The polarity in the intestine is marked by F-actin–rich brush border on the apical side and E-cadherin on the basolateral side. Immunofluorescent stainings showed disrupted polarity in organoids cultured in Matrigel already after 10 days. In contrast, organoids in microwells seem to preserve distinct apico-basolateral borders for the whole culture period. On the other hand, qPCR and stainings for proliferation and differentiation revealed similar expression patterns for organoids in both culture systems. These data confirm the applicability of our microwell platform to intestinal organoid culture and pave the way towards simplified micromanipulation, automated and high-resolution imaging, dynamic culture of organoids and defined exposure of organoids to soluble compounds.

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Abstract **#585**

BIOMOLECULE IMMOBILIZED 3D PRINTED NANOHYBRID SCAFFOLDS FOR ACCELERATED BONE TISSUE REGENERATION

Sanjoy Kumar GhoraiP[1], Trina Roy[2], Santanu Dhara[2], Santanu Chattopadhyay[1]

[1] *Rubber Technology Centre, Indian Institute of Technology Kharagpur*

[2] *School of Medical Science and Technology, Indian Institute of Technology Kharagpur*

Corresponding author's email: sanjoypst07@gmail.com

Critical bone defects with sluggish rate of auto-osteoconduction and imperfect reconstruction urge to develop an alternative approach for regeneration of bone. Tissue engineering for bone regeneration signifies an innovative way to overcome this problem and creates an alternate for bone tissue substitute. Polymer based nanohybrid materials have been widely used for bone tissue regeneration because of their tuneable physicochemical properties and excellent osteoconductive bioactivity. Amongst different fabrication techniques, 3D printing technique is obviously most efficient and advanced way to fabricate the osteoconductive scaffold with control porous structure.1 In the current article, the synthesized polyurethane-urea (P12) based nanohybrid scaffold is fabricated by 3D printing technique by incorporating osteoconductive nanomaterial, titanium phosphate adorned nanohydroxyapatite (TP/nHA).2 The scaffold has been printed at ambient temperature using high viscous polymer slurry in THF. After fabrication, the surface of the scaffold has been modified with the immobilized biomolecule polydopamine (PDA).3 The surface modification of the scaffold has been assessed through AFM, FESEM and XPS study. The incorporated TP improved the physico-mechanical properties of the scaffold drastically through the dative bone formation between the vacant d orbital of the titanium atom and the lone pair electron of amide linkage of the polyurethane-urea. The introduced hybrid nanomaterial within the scaffold also promoted the polydopamine coating by the electrostatic attraction between the positive charged titanium ion and the negative charged hydroxyl group of dopamine. In vitro and in vivo assessment indicates the superior osteogenic bioactivity of the PDA immobilized polyurethane-urea based 3D printed scaffolds without exhibiting any toxic effects.

Keywords

3D printed scaffolds; Polydopamine immobilization; Bone regeneration

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Abstract **#586**

DECELLULARIZED MATRICES OF MESENCHYMAL STROMAL CELLS CULTURED UNDER 20 AND 5% O2 DIFFERENTIALLY REGULATE CELL FUNCTIONS AFTER RECELLULARIZATION

Diana Matveeva[P][1], Sergei Buravkov[2], Elena Andreeva[1]

[1] *Institute of Biomedical Problems of Russian Academy of Sciences, Moscow, Russia*

[2] *Lomonosov Moscow State University, Moscow, Russia*

Corresponding author's email: dianamatv516@gmail.com

The decellularized extracellular matrix (dcECM) is attractive as a source of biological scaffolds for tissue reconstruction. Mesenchymal stromal cells (MSCs) are key components of connective tissue and produce specific ECMs depending on specific tissue milieu. The O2 levels are crucial for microenvironmental regulation of MSC functions. Here we prepared and characterized dcECMs from MSCs permanently cultured under 20% or 5% O2.

Dense monolayers human adipose-derived MSCs were decellularized with 0.5% Triton-X100/20 mM NH4OH/PBS. Scanning electron (SEM) and confocal microscopy were used to examine dcECM morphology. To test functional activity (adhesion and osteo-differentiation), dcECM from 20% O2 (20-dcECM) or 5% O2 (5-dcECM) were recellularized with MSCs at 20% O2. Collagen I coating was used for comparison.

According to SEM, under 5% O2 dcECMs were formed by dense network of thin intersecting fibers, while membrane-like structures without separation into individual fibrils were noted at 20% O2. The efficiency of MSC adhesion was higher on 5-dcECMs after recellularization. At early steps of adhesion, dish-shaped MSCs were prevailed on collagen, most of MSCs on dcECMs had numerous pseudopodia. Both types of dcECMs effectively supported the MSC expansion. Osteo-differentiation of MSCs was more pronounced on dcECMs than on collagen. The spontaneous activity of alkaline phosphatase was 2 times lower on 5-dcECMs, this effect was canceled upon osteoinduction.

The data demonstrate the role of local milieu in regulation of capacities of MSC-derived dcECM when dcECMs are applied as biocompatible coatings for scaffolds.

This work was supported in part by Programm #19 of Presidium of RAS.

Abstract **#587**

INTER-DONOR VARIABILITY EVALUATION OF HUMAN CELL-ASSEMBLED EXTRACELLULAR MATRICES

Fabien Kawecki[P][1], Maude Gluais[1], Stéphane Claverol[2], Nathalie Dusserre[1], Todd McAllister[3], Nicolas L'Heureux[1]

[1] *Univ. Bordeaux, INSERM, BIOTIS, U1026, F-33000 Bordeaux, France.*

[2] *Centre de Génomique Fonctionnelle, Plateforme Protéome, Université de Bordeaux, 33000, Bordeaux, France.*

[3] *Fountain Therapeutics, Culver City, CA, USA.*

Corresponding author's email: fabien.kawecki@inserm.fr

Many laboratories have developed tissue-engineered approaches based on the ability of mesenchymal cells to assemble in vitro their endogenously-secreted extracellular matrix. However, patient-to-patient variability of this cell-assembled extracellular matrix (CAM) production can be a hurdle for clinical applications. This study aimed to evaluate this variability as well as parameters that drive CAM production. To this end, human CAM sheets were produced using different primary dermal fibroblast populations obtained from 21 donors/patients requiring arteriovenous shunt.

Results demonstrated that the strength, the thickness, and the hydroxyproline content of the CAM sheets varied between donors by 33% (coefficient of variation), 19%, and 24%, respectively. Another objective was to characterize the CAM matrisome to better understand its relation with the CAM properties. The CAM sheet strength showed moderate and strong positive correlations with the CAM thickness and hydroxyproline quantity, respectively. A detailed CAM matrisome characterization was performed by mass spectrometry and confirmed the inter-donor variability in term of CAM protein composition. Data also revealed that the CAM strength correlates with collagen alpha-1(I) chain abundance. The CAM thickness showed strong correlations with fibrillin-1, dermatopontin, and peroxidasin, which are involved in collagen fibril formation and stabilization. In addition, the CAM hydroxyproline quantity intensely correlated with proteoglycans (e.g. decorin) and ECM regulators (e.g. serpin H1) involved in collagen fibril biosynthesis.

Finally, this study formally evaluates the CAM inter-donor variability in a clinical manufacturing context. Furthermore, the detailed CAM composition characterization identified molecular predictor of the CAM properties and possible targets for improving CAM strength.

Keywords

Cell-Assembled Extracellular Matrix; Inter-Donor Variability; Human Fibroblast

Abstract **#588**

OPTIMISED CELL SEEDING STRATEGIES INFORMED BY COMPUTATIONAL MODELS TO ADVANCE ENGINEERED NERVE REPAIR CONSTRUCT DESIGN

Despoina Eleftheriadou[1,2,3], Maxime Berg[1,3], James B Phillips[1,2], Rebecca J Shipley[1,3]

[1] *UCL Centre for Nerve Engineering, London, UK*

[2] *Department of Pharmacology, UCL School of Pharmacy, London, UK*

[3] *UCL Department of Mechanical Engineering, London, UK*

Corresponding author's email: rmhklef@ucl.ac.uk

Recent advances in peripheral nerve injury treatments have focused on tissue engineering approaches which combine therapeutic cells and biomaterials. However, the design of bioengineered nerve repair constructs (NRCs) has so far been informed predominantly by experiments, which can be time-consuming and result in substandard designs. Mathematical modelling offers a cost- and time- effective way of studying the behaviour of therapeutic cells in NRCs and allows us to make better-informed predictions about their overall efficacy.

Values of unknown parameters that describe the behaviour of differentiated human neural stem cells under a range of physiologically-relevant conditions were initially calculated based on experimental observations obtained from a well set-up. These were subsequently used in a multiphysics mathematical model of an NRC design2. We then simulated a variety of cell seeding strategies and temporal changes on cell survival and vascular endothelial growth factor (VEGF) release. Finally, based on the simulation results, we examined different fabrication techniques that would enable successful patterning of cells within engineered NRCs.

Simulation results indicate that the use of lower seeding cell densities could lead to higher viability of cells within the first few days post-implantation. Moreover, non-uniform seeded cell distributions could enhance overall cell survival and promote the creation of VEGF gradients to encourage neo-vascularisation. Based on the model predictions, 3D neural tissue replacements with optimised cellular distributions were manufactured using microfabrication techniques.

The results of this work highlight how interdisciplinary approaches can accelerate the design of nerve repair constructs for future translation into the clinic.

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Abstract **#589**

STAKEHOLDER PERSPECTIVES ON DEALING WITH ETHICAL CHALLENGES IN STEM CELL RESEARCH

Lars Assen[P][1], Karin Jongsma[1], Rosario Isasi[2], Annelien Bredenoord[1]

[1] Julius Center for Health Sciences and Primary Care, Department of Medical Humanities, University Medical Center Utrecht

[2] Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine

Corresponding author's email: l.s.assen@umcutrecht.nl

Induced Pluripotent Stem Cells (iPSCs) have become an integral part of biomedical research in the last fourteen years. iPSCs overcome some ethical challenges inherent to embryonic stem cells; however, it has ethical challenges of its own. Ethical challenges in the research phase that have been described in the literature concern the collection, storage and use of donor material, and questions about ownership. Moving towards clinical translation, ethical challenges such as undesirable side-effects, teratoma formation, stem cell tourism and costs of therapy could arise (2,3).

Yet, little is known about the perspectives and experiences of stakeholders such as iPSC-researchers, regulators and (former) patients concerning how to deal with these challenges. Therefore, in order to get these insights, we have conducted focus groups with stem cell researchers, regulators and (former) patients with chronic lower back pain. The results of these focus groups have been analysed. In this paper the results of these focus groups will be discussed. The results shed light on amongst others what the role of policy should be in dealing with ethical challenges and what researchers need to be able to deal with the ethical challenges of iPSC-research.

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Abstract **#590**

CHARACTERISING THE PROTEOME OF THE FOETAL NOTOCHORDAL CELL: IMPLICATIONS FOR INTERVERTEBRAL DISC DEGENERATION AND REGENERATION

Stephen M Richardson[P][1], Lizzy Ward[1], Richard D Unwin[1], Anna L Tierney[1], Andrew W Doswey[2], Judith A Hoyland[1]

[1] *University of Manchester*

[2] *University of Bristol*

Corresponding author's email: s.richardson@manchester.ac.uk

Loss of large, vacuolated notochordal cells (NCs) from the human intervertebral disc (IVD) is thought to initiate degeneration and associated back pain. It is therefore hypothesised that implantation of NCs may halt or reverse degeneration and thus relieve back pain. However, NCs are lost in early childhood, therefore iPSCs differentiation to NCs offers a clinically-viable cell source. Here we aimed to characterise the proteomic profile of the human foetal NCs, versus surrounding annulus fibrosus (AF) cells. FACS sorting for CD24, a known NC marker, was used to isolate NC (CD24+) and AF (CD24-) cells from microdissected human IVDs (14-15 weeks post-conception, n=3). Following iTRAQ isobaric tagging, TripleTOF mass spectrometry and subsequent bioinformatics, differential protein expression was validated by immunofluorescence. Our study revealed 100 up-regulated and 8 down-regulated proteins in the CD24+ population ($-1.5 \geq FC \geq 1.5$, $P \leq 0.1$), including known (e.g. keratins 8 and 19) and novel phenotypic markers. Ingenuity Pathway Analysis (IPA) revealed pathways known to play an important role in NCs and IVD homeostasis, such as NRF2-mediated oxidative stress response and caveolar-mediated endocytosis, and putative upstream regulators known to be active in NCs e.g. TGFβ1 and SMAD3. The same analysis predicted many previously unknown proteins, pathways and regulators to be active or repressed in NCs. Together, these results validate our method as a powerful tool for isolation and proteomic analysis of foetal NC cells and reveal novel proteins and pathways of potential use in the development of future strategies for the study and treatment of IVD degeneration.

Keywords

notochord; degeneration; proteomics

Abstract **#591**

THERMO-RESPONSIVE CHITOSAN HYDROGELS LOADED WITH ANTIBIOTICS WITH SYNERGISTIC ANTIBACTERIAL EFFECTS FOR TREATMENT OF XDR BACTERIA-INFECTED FULL-THICKNESS WOUNDS

Zahra Aliakbar Ahovan[P][1], Sadjad Khosravimelal[2,3,4], Behnaz Sadat Eftekhari[5,6], Soraya Mehrabi[2,7], Ali Hashemi[1], Mazaher Gholipourmalekabadi[2,4]

[1] *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2] *Cellular and Molecular Research Centre*

[3] *Department of Medical Biotechnology, Faculty of Allied Medicine*

[4] *Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*

[5] *Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran.*

[6] *Department of Physiology and Institute for Medicine and Engineering, University of Pennsylvania, USA.*

[7] *Department of Physiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

Corresponding author's email: ahovanzahra@gmail.com

The high prevalence of antibiotic resistance is now considered a major global challenge. Conventional therapies for treating skin wound infection with extensively drug-resistant (XDR) bacteria are likely to be inadequate due to the lack of efficient antibiotics. In this study, we have evaluated the optimized concentration of Colistin and Meropenem as a combined drug. Antibiotic-loaded thermo-responsive chitosan (TCTS) hydrogel was evaluated as a potential formulation to protect full-thickness wounds infected with XDR bacteria isolated from burn patients, both in vitro and in vivo in a rat model. Antibacterial activity of the TCTS hydrogel against standard strain and clinical isolates of Acinetobacter baumannii and Pseudomonas aeruginosa, cytobiocompatibility for Hu02 fibroblast cells, chemical structures, morphologies, degradation rate, swelling ratio, and release behavior were determined in vitro. The results of disk diffusion assay showed that the antibacterial activity of TCTS hydrogel is optimal at concentration of 8 µ/ml and 4 µ/ml for Colistin and Meropenem, respectively. The TCTS hydrogel itself didn't exhibit antibacterial effect. Furthermore, MTT showed no cytotoxicity of TCTS hydrogel against fibroblasts. In vivo study showed significant acceleration of wound healing, re-epithelialization, wound closure, collagen disposition, up-regulated CD31, and decreased colony count in the TCTS hydrogel-loaded optimized concentration of antibiotics group compared with TCTS hydrogel and gauze as control groups. The results of this study showed great promise for drug-loaded TCTS hydrogel as a wound dressing for managing wounds infected with XDR bacteria.

Keywords

Thermos-responsive Hydrogel; Antibiotic resistance; XDR bacteria; Colistin; Meropenem

Abstract **#592**

MICROFLUIDIC PLATFORM FOR MESENCHYMAL STEM / STROMAL CELL DERIVED EXTRACELLULAR VESICLE ANALYSIS

Damien King$_P$[1], Adam Murphy[1], Josh Fallon Doran[1], Raphaela Ferreira[1], Rosheen Spier[1], Zoe Deignan[1], Abidemi Olayinka[2], Paul A Cahill[2]

[1] *Fraunhofer Project Centre for Embedded Bioanalytical Systems FPC@DCU*

[2] *Vascular Biology & Therapeutics, School of Biotechnology, DCU*

Corresponding author's email: damien.king@dcu.ie

Within the EVPro project the design for manufacture optimisation of a microfluidic lab on a disc (LoaD) platform for size sorting, antibody based classification and retrieval of extra cellular vesicles (EVs) derived from mesenchymal stem / stromal cell (MSCs) has been investigated. EVs generated from MSCs grown under static cell culture conditions (multilayer culture flasks) and using a hollow fibre bioreactor (HFBR) have been examined.

The innovative LoaD device has been developed for capture and differentiation of the EVs based on size categorization (in the region <200nm, with separations in the sub-regions of 100nm – 70nm, 70nm – 50nm and <50nm). Once captured and categorized by size, the EVs will be examined for their expression levels of the relevant therapeutic biomarkers (CD9, CD63 and CD81). Filter membrane configurations to perform the size based classifications of the generated EVs have been reviewed and finalized. LoaD designs using rapid prototyping, xurography and milling have been generated and upon completion of fluidic validation will be transferred for generation of an injection moulding tool for pilot scale LoaD manufacture.

The finalized device will serve as an at line quality check tool for EV production derived from MSC culture methods. The device will also assist the EVPro project partners with GMP compliance and up scaling to a GMP facility for EV production.

This project has received funding from the European Union's Horizon 2020 research and innovation action under grant agreement No. 814495-EVPRO (www.evpro-implant.eu)

Abstract **#593**

NON-SPECIFIC INTERNALIZATION OF IMMUNOMAGNETIC MICROPARTICLES BY CONTAMINATING STROMAL CELLS IMPEDES THE EXPANSION OF HUMAN ADIPOSE TISSUE-DERIVED MICROVASCULAR ENDOTHELIAL CELLS

Jeremy Antonyshyn[P][1,2], Meghan McFadden[1,2], Anthony Gramolini[1,2], Stefan Hofer[1,3], Paul Santerre[1,2]

[1] *University of Toronto, Toronto, Canada*

[2] *Ted Rogers Centre for Heart Research, Toronto, Canada*

[3] *University Health Network, Toronto, Canada*

Corresponding author's email: jeremy.antonyshyn@mail.utoronto.ca

Background. Adipose tissue is an accessible source of endothelial cells (AMVECs) for vascular tissue engineering. However, their cultures are frequently overgrown by residual stromal cells (ASCs) from the sorting procedure. The challenge in expanding AMVECs was investigated to facilitate their procurement in large quantities with high purity.

Methods. CD45–CD31+ AMVECs were isolated using immunomagnetic microparticles (IMPs). Efficacy was evaluated by flow cytometry (FCM). IMP localization was assessed by confocal microscopy. Prevalence of ASCs with bound/internalized IMPs was determined using FCM. Proteomics was used to identify an AMVEC-specific immunophenotype. Anti-CD31 and anti-CD93 IMPs were characterized by Coulter counter, and their binding/internalization by ASCs was compared by FCM.

Results. AMVECs were 98.3±1.0% CD45–CD31+, a 34-fold enrichment (2.9±0.6%; $p<0.05$). However, 85% of cultures were overgrown by CD45–CD31– ASCs, despite sequential enrichments. ASCs exhibited membrane-bound and internalized anti-CD31 IMPs. Percentage ASCs with membrane-bound IMPs decreased over time (0.33hrs: 12.1±3.2% vs. 7dys: 4.4±2.1%; $p<0.05$), while IMP internalization increased over time (0.33hrs: 5.0±2.8% vs. 7dys: 26.3±7.5%; $p<0.05$). Proteomics found ASCs and AMVECs shared conventional immunophenotypic markers, including CD31, and identified CD93 as being AMVEC-specific. Anti-CD31 and anti-CD93 IMPs exhibited similar sizes (5.0±0.6 vs. 5.0±0.5 µm, respectively), and their binding (3.8±0.5% vs. 3.9±0.9%, respectively) and internalization (8.6±5.8% vs. 7.6±5.2%, respectively) by ASCs was comparable after 48hrs in culture.

Conclusion. Non-specific internalization of IMPs by residual ASCs from the sorting procedure precludes the sequential enrichment of contaminated AMVEC cultures. Exclusion of IMPs from cultures will reduce the prevalence of their internalization by ASCs and, thereby, enable sequential enrichments.

Keywords

adipose tissue-derived microvascular endothelial cells; adipose tissue-derived stromal/stem cells; microparticles

Abstract **#594**

LASER-ENGRAVED AURICULAR CARTILAGE SCAFFOLDS: THE NEXT STEP ON RECELLULARIZATION

Isabel Casado Losada[P][1,2], Cornelia Schneider[2], Barbara Schädl[2], Johann Zehetner[3], Sylvia Nürnberger[1,2]

[1] *Department of Orthopedics and Trauma-Surgery, Division of Trauma-Surgery, Medical University of Vienna, Vienna, Austria*

[2] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology. Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[3] *Research Centre for Microtechnology, University of Applied Sciences, Dornbirn, Austria*

Corresponding author's email: isabel.casadolosada@meduniwien.ac.at

Cartilage is a very complex tissue with poor regenerative capacity due to its avascularity and absence of nerve and lymphatic innervation. Among the different strategies to regenerate cartilage defects, the use of decellularized scaffolds from native cartilage has become of great interest, but the remaining dense matrices are difficult to recellularize. Two strategies have been developed recently by our group to perforate the dense matrix: laser-engraving of articular cartilage and enzymatic treatment of auricular cartilage. Within this study, we want to combine both strategies, comparing the efficiency of laser-engraving auricular cartilage with a CO2 or a femtosecond laser, and further test the performance of chondrocytes of different age. Scaffolds were prepared from cartilage discs from auricular cartilage from bovine ears. They were, subsequently, laser engraved and chemically treated with different formulations containing elastin, pepsin, and HCl. For recellularization, human chondrocytes from different donors were labeled with vital dyes, pooled, and cultured under dynamic conditions. The scaffolds lasered with femtosecond laser showed better integrity than those with CO2. Furthermore, repopulation of the laser incised auricular scaffolds was achieved within a few days and was less influenced by the laser pattern than the chemical treatment. Besides, donor age influenced the speed of cell distribution within the scaffold. Overall, this study proved that laser-engraving is a feasible efficient method to improve cell infiltration in auricular cartilage scaffolds making them an ideal chondrogenic environment for the treatment of cartilage defects.

Abstract **#595**

OPTICAL FIBER-BASED APPROACH TO CREATE MICROFLUIDICS PLATFORMS: A STRAIGHTFORWARD SOLUTION FOR THE PRODUCTION OF MICROGEL BIOINKS

Alessandro Cianciosi[1], Jürgen Groll[1], Tomasz Jüngst[1]
[1] *Department of Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, University of Würzburg*
Corresponding author's email: alessandro.cianciosi@fmz.uni-wuerzburg.de

Biofabrication techniques bear the potential to generate biologically functional products [1]. Recently, great attention has been put on the development of new bioinks [2]. A promising alternative to conventional inks are cell-laden jammed microgel inks [3]. The microgels, which are the crucial building blocks for this bioink type, can be obtained using droplet-generating microfluidic devices. Despite the great advances in the fabrication of microfluidics chips, most of the approaches are still time consuming and expensive [4]. This study proposes a novel, cost effective, fast and versatile process for the fabrication of microchannels based on an optical fiber (OF)-coupled LED light. The OF is integrated with a commercially available FDM printer, which controls its movement complying with a predetermined microfluidic design. This method relies on selective photo-patterning of a thin layer of light-sensitive resin deposited onto a cover slip using the OF as light-source. An elastomer (PDMS) is used for molding and the microfluidic chip is obtained after plasma bonding of the PDMS to a cover slip. A key aspect of the study is analysing the resolution of the microchannels, which is demonstrated to be influenced by OF speed, light intensity and resin layer thickness. The microfluidic chips are used to generate homogeneous cell-laden microgels from hydrogel precursors which can be jammed into microgel bioinks for extrusion-based bioprinting. In conclusion, this study shows an easy and straightforward approach for the fabrication of microfluidics platforms, which enables the production of microgel-based bioinks for extrusion-based bioprinting.

Keywords
Microfluidics; Optical fibers; Microgel bioinks

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Abstract **#596**

IDENTIFICATION OF A PROXIMAL TUBULE EPITHELIAL CELL LINE CIPTEC-OAT1 AS A NOVEL SENESCENCE MODEL

Yi Yang$_P$[1], Milos Mihajlovic[1], Floris Valentijn[2], Tri Q Nguyen[2], Roel Goldschmeding[2], Rosalinde Masereeuw[1]

[1] *Division of Pharmacology, Department of Pharmaceutical Sciences, Utrecht University, The Netherlands*

[2] *Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands*

Corresponding author's email: y.yang@uu.nl

Background

Accumulating evidence suggest that cellular senescence influences kidney fibrosis. Senescent cells secrete senescence-associated secretory phenotype (SASP) markers that can be involved in diverse signaling pathways, influencing kidney fibrosis. Recent studies have focused on clearing senescent cells as treatment option for kidney fibrosis. Our previous study suggested that ciPTEC-OAT1 is a suitable model for studying kidney diseases. Here we investigated whether ciPTEC-OAT1 can also be a potential senescence model.

Methods

CiPTEC-OAT1 cells were seeded into 6-well format plates and were grown at 33℃, then transferred to 37℃ for maturation and cultured for 12 days. The culture medium and cell lysate were collected on day 3, 6, 9 and 12, and used for detecting senescence phenotypes, including apoptosis markers, DNA damage markers, and common SASP factors on the gene and protein level.

Results

Long-time (>6 days) maturation at 37℃ affects the senescence phenotypes. Compared to non-heat transferred cells, heat transferred cells showed an upregulation of p21 ($p<0.001$), PAI-1 and IL-6 ($p<0.05$), and a downregulation of Lamin B1 ($p<0.01$) on the mRNA level. On the protein level heat transferred cells showed an upregulation of Bcl-2 ($p<0.01$) and Bax ($p<0.05$), and a downregulation of Mcl-1($p<0.05$), Puma ($p<0.05$), BID ($p<0.05$) and Bim ($p<0.05$), suggesting anti-apoptosis pathway is activated. Further, common SASP factors like IL-6, TNF-α and TGF-β ($p<0.05$) were also upregulated on the protein level during maturation.

Conclusion

The results suggest that ciPTEC-OAT1 obtain senescence phenotype over time under maturation at 37℃. Future studies will be directed to evaluate senolytics-response in this senescence model.

Abstract **#599**

EPIGENETIC REPROGRAMMING ENHANCES THE THERAPEUTIC EFFICACY OF OSTEOBLAST-DERIVED EXTRACELLULAR VESICLES FOR BONE REGENERATION

Kenny Man$_P$[1], Mathieu Brunet[1], Maria Fernandez-Rhodes[2], Soraya Williams[2], Owen Davies[2], Angelica Federici[3,4,5], David Hoey[3,4,5], Sophie Cox[1]

[1] School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom.

[2] School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom.

[3] Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland.

[4] Dept. of Mechanical, Manufacturing, and Biomedical Engineering, School of Engineering, Trinity College Dublin, Ireland.

[5] Advanced Materials and Bioengineering Research Centre, Trinity College Dublin & RCSI, Ireland.

Corresponding author's email: k.l.man@bham.ac.uk

For bone regeneration, there is great precedence to develop instructive acellular technologies that circumvent limitations with the translation of cell-based therapies. Extracellular vesicles (EVs) derived from osteoblasts enhanced stem cell mineralisation compared to BMP-2 (1, 2). Regulating the cell's epigenetics through histone deacetylase (HDAC) inhibition enhances their differentiation potential. Therefore, this study investigated altering osteoblasts epigenetic functionality via the HDAC inhibitor Trichostatin A (TSA) on promoting osteoblast-derived EVs potency.

TSA effect on osteoblast epigenetic functionality and mineralisation was determined by quantifying HDAC activity and calcium deposition. EVs were isolated from untreated/TSA treated osteoblasts for 2 weeks. EV size and concentration were defined using nanoparticle tracking analysis. EVs microRNA expression was evaluated using microarray analysis. Osteogenic differentiation of human bone marrow stromal cells (hBMSCs) cultured with untreated (MO-EVs)/TSA treated osteoblast-derived EVs (TSA-EVs) was evaluated by qPCR, biochemistry and histological analysis.

TSA significantly reduced osteoblast HDAC activity and enhanced calcium deposition when compared to untreated cells. The quantity of EVs generated, in addition to their protein content and size correlated with the degree of osteoblast differentiation. TSA-EVs accelerated hBMSCs proliferation and migration compared to MO-EVs. Importantly, TSA-EV treatment significantly upregulated hBMSCs osteoblast-related gene/protein expression (ALP, Col1a, BSP1, OCN) and promoted extracellular matrix mineralisation when compared to MO-EV treatment during osteogenic culture. Microarray analysis revealed TSA-EVs were enriched with several pro-osteogenic microRNAs involved in regulating mechanisms such as "endocytosis" and "Wnt signalling pathway".

Together, epigenetic reprogramming provides a novel engineering approach to enhance the therapeutic efficacy of osteoblast-derived EVs for bone augmentation strategies.

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Abstract **#600**

A NOVEL MESOSCALE HUMAN MUSCLE-TENDON-BONE INTERFACE MODEL FOR THE STUDY OF MUSCULOSKELETAL DISEASES

Valerio Luca Mainardi[1,2,3], Sushila Maharjan[3], Giuseppe Talò[4], Simone Bersini[1], Christian Candrian[1], Gabriele Dubini[2], Matteo Moretti[1,4], Yu Shrike Zhang[3]

[1] *Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale (EOC), 6900 Lugano, Switzerland*

[2] *Laboratory of Biological Structures Mechanics (LaBS), Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, 20133 Milan, Italy*

[3] *Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 02139 Cambridge, MA, USA*

[4] *Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, 20161 Milan, Italy*

Corresponding author's email: valerio.mainardi@eoc.ch

Although during physio-pathological processes there is an extensive crosstalk among musculoskeletal tissues, traditionally the single components have been studied separately1. To better investigate the pathogenesis of specific musculoskeletal diseases, it is necessary to develop advanced in-vitro models able to replicate the architecture and interface of musculoskeletal tissues through the integration of multiple biological components in the same device2.
Here, a mesoscale human muscle-tendon-bone interface was created using a perfusable multi-chamber device. Computational simulations were performed to optimize the geometry of the device and minimize (<10%) the level of mixing of the different media used to culture the different tissues, while allowing an adequate fluid dynamic stimulation of the biological construct (mean fluid velocity 7mm/s, mean wall shear stress 25mPa). 12 different configurations were tested using a syringe pump to perfuse the device. A digital light processing-based bioprinter was applied to produce the biological construct orienting muscle and tendon fibers towards the main direction of the flow and replicating the bone macroscopic porosity3. A single process was used to realize the continuous biological construct, embedding in gelatin/methacryloyl-based hydrogel myoblasts, tendon fibroblasts, and osteogenically differentiated mesenchymal stem cells4.
Multi-cellular constructs structurally and functionally connected were developed stimulating each tissue with the proper conditions to optimize their maturation. Subsequently, a model of tendinous inflammation, observable in every tendinopathy, was developed assessing its propagation towards muscle and bone. The proposed model represents an innovative and versatile tool to investigate all those physio-pathological mechanisms in which the crosstalk between different tissues plays a dominant role.

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Abstract **#601**

SECRETORY AND TRANSCRIPTIONAL ACTIVITY OF RAT BONE MARROW OSTEOPROGENITORS AFTER 14-DAY HINDLIMB SUSPENSION

Elena Markina[P][1], Milena Daniel[1], Ludmila Buravkova[1]

[1] SSC RF – IBMP RAS, Moscow

Corresponding author's email: goncharova-tim@list.ru

It is known that during space flight and under simulation of microgravity effects, negative changes in bone tissue are observed. The RANK/RANKL/OPG system and proinflammatory mediators (IL-1,-6,-11, TNF-a) play a significant role in bone remodeling processes. Studies of changes in the potential of bone marrow osteoprogenitors in microgravity are relevant of understanding of bone regenerative processes.

The goal of our work was to study the effect of 14-day suspension and subsequent 14-day recovery on the secretory and osteoprogenitors transcriptomic activity. For this purpose, the rat bone marrow cultured stromal cells secretom was investigated. Gene transcription in MSCs was determined by quantitative PCR.

After hindlimb suspension, the SPP and Cxcl12 genes expression in MSCs was down-regulated and osteoprotegerin (OPG) and sclerostin (SOST) concentration in cultured medium was decreased. At the same time the level of IL-6 and TNF-a was increased. After readaptation, the expression SPP, Cxcl12 genes was the same the same as in the control group. The OPG concentration was high and the SOST secretion remained low. At the same time, the IL-6 and TNF-a concentration in the culture medium was decreased up to control level.

Thus, the support unloading during 14 days leads to an inhibition of the bone marrow osteoprogenitor activity, which determined the processes of bone remodeling. This was accompanied by decreasing of their osteoplastic function. After 14-day readaptation, osteogenic bone marrow progenitor activity recovered.

This work was supported by the basic research program of the SSC IMBP RAS 65.3 and the RFBR grant 19-015-00206

Abstract **#603**

MECHANICAL PROPERTIES OF RABBIT ACELLULAR DERMIS VERSUS HUMAN ACELLULAR DERMIS FOR USE IN SKIN GRAFTING

Maryam Mousavi Khatat$_P$[1], Leila Roshangar[1,2,3], Keyvan Moharramzadeh[4], Jafar Soleimani Rad[1,3], Ahmad Mehdipour[1]

[1] *Department of Tissue Engineering, Faculty of Advanced Biomedical Sciences, Tabriz University of Medical Sciences*

[2] *Stem Cells Research center, Tabriz University of Medical Sciences*

[3] *Department of Anatomical Sciences and Histology, Faculty of Mediicine, Tabriz University of Medical Sciences*

[4] *University of Sheffield*

Corresponding author's email: khatat.mm@gmail.com

Objective: In many clinical situations, the need for skin grafting is raised. One method of providing connective tissue is xenograft or allograft acellular Dermis. If the specifications of the xenograft meet the requirements for the transplant operation, it is superior to the allograft; one of these capabilities is the mechanical properties of alternative skin tissue. This study aimed to investigate the mechanical properties of Lapine cellular Dermis tissue versus the mechanical properties of human cellular skin for extensive production and tissue replacement applications.

Methods: Human and rabbit skin samples were divided into two groups of control and intervention after transfer to the laboratory. In the intervention groups used the same acellularization protocol for Lapine tissue and for human tissue. The control groups did not receive any treatment. After removing the cells from the tissues, the necessary incisions and molding were performed on the samples, and all groups were subjected to a tensile test with the Instron 5566, USA, and the amount of tissue tension compared. The rate 5mm/min.

Results: The results showed that in human samples, the difference in tensile properties between the intervention group and the control group was significant ($p<0.001$). This difference was also significant in the rabbit samples ($p<0.001$). In addition, there was a significant difference between the human intervention group and Lapine ($p<0.001$).

Conclusion: Despite the significant differences between the control and intervention groups and also between the intervention groups, the values obtained are still acceptable as a skin alternative according to previous studies.

Abstract **#604**

MICRO ENGINEERED PLATFORMS TO STUDY AND CONTROL EMBRYONIC MORPHOGENESIS USING IN VITRO STEM CELL-BASED MODELS

Pinak Samal[1], Clemens van Blitterswijk[1], Roman Truckenmüller[1], Stefan Giselbrecht[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands*

Corresponding author's email: p.samal@maastrichtuniversity.nl

Stem-cell-based in vitro models of morphogenesis can help shed light on the mechanisms involved in embryonic patterning. These models have been previously showcased by others using traditional cell culture platforms which have limited single-cell imaging capabilities and control over the biological system. Using micro engineered tools can help in precise control over the culture microenvironment and real-time data acquisition. This can in turn aid in further understanding the underlying mechanisms involved in tissue formation. Here, we describe thin polymer based microdevices, which were fabricated using microthermoforming, and show their application to study in vitro morphogenesis(1). A pipeline consisting of open-source software to quantify 3D cell movement within stem cell aggregates using automated image acquisition was developed. Finally, the platform was applied to study and control morphogenesis in 3D cultures of the P19C5 mouse cell line and mouse embryonic stem cells (mESCs), which both show symmetry breaking and axial elongation events similar to early embryonic development. The results obtained in this study indicate that polarization and elongation of the mESC aggregates are dependent on directed cell migration. The platform was applied for testing the effect of the drug Latrunculin A on aggregate morphogenesis and exposure to the drug was found to impair cell migration within the aggregate. Overall, this method paves way for high-throughput studies and drug testing applications using in vitro models, which mimic embryonic development.

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Abstract **#605**

INTERLEUKIN-1B AND CATHEPSIN-D MODULATE THE TERMINAL COMPLEMENT COMPLEX FORMATION IN HUMAN DISC TISSUE CULTURES

Graciosa Q Teixeira[P][1], Zhiyao Yong[1], Amelie Kuhn[2], Jana Riegger[2], Michael Ruf[3], Anita Ignatius[1], Rolf Brenner[2], Cornelia Neidlinger-Wilke[1]

[1] *Institute of Orthopaedic Research and Biomechanics, Ulm University, Ulm, Germany*

[2] *Division for Biochemistry of Joint and Connective Tissue Diseases, Department of Orthopedics, Ulm University, Ulm, Germany*

[3] *Spine Center, Karlsbad-Langensteinbach, Germany*

Corresponding author's email: graciosa.teixeira@uni-ulm.de

Introduction: The formation of the terminal complement complex (TCC), a complement system activation product that triggers inflammation and cell lysis, was previously identified in degenerated discs, being correlated with their degeneration degree [1]. However, it is unclear which molecular factors play a role in complement activation during disc degeneration (DD). Therefore, we investigated possible triggers of TCC formation in DD.

Methods: Disc biopsies were collected from adolescent idiopathic scoliosis (AIS, n=8, age 16±3) and DD (n=11, age 56±15) patients with ethical approval and informed consent. Standardized tissue punches from nucleus pulposus (NP), annulus fibrosus (AF) and endplate (EP) were separately cultured. Tissues were stimulated with medium containing 5% human serum alone or supplemented with interleukin-1β (IL-1β, 10ng/mL) or cathepsin-D (0.5ug/mL). Serum-free medium served as control. TCC formation and CD59 (TCC inhibitor) expression were analyzed by immunohistochemistry. Statistics: one-way ANOVA.

Results: No differences were found between scoliosis and DD patients. IL-1β stimulation led to lower percentage of TCC+ cells in AF and EP ($p<0.05$), whereas cathepsin-D significantly increased TCC deposition in NP ($p<0.01$). The percentage of CD59+ cells significantly increased in AF and NP after cathepsin-D stimulation ($p<0.05$).

Conclusion: Interestingly, IL-1β led to less TCC deposition in AF and EP, indicating that it may influence DD it via a negative feedback mechanism. In contrast, cathepsin-D, an important player in osteoarthritis, but poorly described in DD, triggered TCC deposition in NP. Mechanistic studies are ongoing, but these results suggest a functional relevance of IL-1β and cathepsin-D in modulating TCC formation in DD.

Keywords

disc degeneration; complement system activation; immunomodulation

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Abstract **#607**

ULTRA-HIGH THROUGHPUT PRODUCTION OF HOLLOW MICROMATERIALS BY IN-AIR MICROFLUIDICS FOR TISSUE ENGINEERING APPLICATIONS

Bas van Loo[1], Nuno Araújo-Gomes[1], Vincent de Jong[1], Tom Kamperman[1], Jeroen Leijten[1]
[1] *Department of Developmental BioEngineering, TechMed Centre, University of Twente, the Netherlands*
Corresponding author's email: s.r.vanloo@utwente.nl

Introduction:
Hollow micromaterials such as hydrogel microcapsules are gaining attention for the production of cellular aggregates and drug delivery purposes.[1] Although microfluidic production of microcapsules offers several advantages over conventional micro-aggregate forming techniques, current approaches suffer from low production throughputs and required use of oils and surfactants, which hinders clinical and industrial applications. To address this challenge, we report on the use of a recently developed platform, named In-Air microfluidics[2], enabling ultra-high throughput production of multiple types of hollow micromaterials in an oil-free and surfactant-free manner.

Methods:
Multiple In-Air microfluidic designs were assessed on their ability to produce hydrogel microcapsules composed of a 10% dextran core and a 0.2% alginate shell. Monodisperse microcapsules with diameters between 80 and 300 µm were produced with production rates as high as 6 ml/min.

Results/discussion:
A two nozzle design effectively produced bulk gels composed of monodisperse hollow compartments. Collecting the flow-through in a stirred collection bath with optimized rotational speed allowed for production of continuous microfibers with regularly spaced hollow compartments. These compartmentalized fibers can form large amounts of consistent and functional 3D microtissues which benefits straightforward retrievability for in vivo applications. Using a three nozzle approach, individual monodisperse single-, double-, and triple-core hollow microgels could be produced in an ultra-high throughput and single-step manner. Microcapsules demonstrated high cell viability and allowed for 3D cellular microtissue formation.

Conclusion:
In-Air microfluidics can be used to fabricate distinct hollow-core micromaterials in a clean and ultra-high throughput manner to facilitate clinical and industrial applications of hollow micromaterials.

Keywords
In-Air Microfluidics; Microcapsules; Cellular micro-aggregates

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Abstract **#608**

STROMAL CELL IMPURITIES UNDERMINE THE TEMPORAL STABILITY OF SMALL DIAMETER VASCULAR GRAFTS ENDOTHELIALIZED WITH HUMAN ADIPOSE TISSUE-DERIVED MICROVASCULAR ENDOTHELIAL CELLS

Jeremy Antonyshyn$_P$[1,2], Stefan Hofer[1,3], Paul Santerre[1,2]

[1] *University of Toronto, Toronto, Canada*

[2] *Ted Rogers Centre for Heart Research, Toronto, Canada*

[3] *University Health Network, Toronto, Canada*

Corresponding author's email: jeremy.antonyshyn@mail.utoronto.ca

Background. Endothelialized vascular prostheses exhibit comparable patency to gold-standard vein grafts[1]. However, patients with acute ischemia cannot tolerate the prolonged endothelialization procedure, which is largely due to the expansion of endothelial cells (ECs) from explanted veins[1]. Adipose-derived ECs (AMVECs) can be readily harvested intra-operatively, but have failed to improve patency[2]. It was hypothesized that residual stromal cells (ASCs) from the sorting procedure undermine the temporal stability of the AMVEC-derived endothelium.

Methods. In vitro models were generated from human adipose tissue. Population doubling times (PDT) of AMVECs and ASCs were characterized. Cells were seeded in pre-defined proportions to re-capitulate different efficacies of AMVEC enrichment. Effect on proliferation was assessed by flow cytometry, and localization by confocal microscopy.

Results. PDT of AMVECs > ASCs (51.3±9.1 vs. 23.6±3.3hrs, respectively; $p<0.05$). AMVEC proliferation was correlated with seeding purity, exhibiting growth inhibitions of 59.5±12.8% and 83.7±7.5% when ASCs comprised 20% and 80% of the seeding density, respectively ($p<0.05$). These disparate growth-kinetics culminated in AMVECs comprising 23.0±17.1% and 1.0±0.8% of the co-cultures after only 4dys ($p<0.05$ vs. 80% and 20% seeding purity, respectively). While pure AMVECs (98.8±0.8% CD31+) integrated into a stable monolayer for >21dys, mixtures of AMVECs and ASCs (1:1) reorganized into microvascular networks in <7dys.

Conclusion. AMVEC-seeded vascular prostheses tend to stenose from diffuse neo-intimal hyperplasia[2]. This may be attributed to poor seeding purities and the disparate growth kinetics of AMVECs and ASCs, which also destabilizes the endothelium. Strategies to obtain pure AMVECs to improve the temporal stability of the endothelium are under investigation.

Keywords

tissue-engineered vascular graft; adipose tissue-derived microvascular endothelial cells; adipose tissue-derived stromal/stem cells

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Abstract **#609**

DESIGN AND DEVELOPMENT OF A NOVEL BIOREACTOR TO ENHANCE THE STRUCTURE AND FUNCTION OF HUMAN TISSUE MODELS

Henry W Hoyle$_P$[1], Stefan A Przyborski[1,2]

[1] *Department of Biosciences, Stockton Road, Durham University, Durham, UK*

[2] *Reprocell Europe, Sedgefield, UK*

Corresponding author's email: h.w.hoyle@durham.ac.uk

The growth of cultured cells is governed by factors within the microenvironment[1]. The dynamic motion of blood through tissue is absent in conventional cell culture and while several systems have attempted to introduce dynamic fluid flow to support cell models in vitro these often have a large footprint, require complex apparatus, are expensive, and have limited scalability.

To overcome these limitations, we have developed a compact benchtop perfusion bioreactor system which allows three-dimensional, centimetre-scale cell constructs to be formed with fluid flow through multiple cell layers maintaining high, homogenously distributed nutrient availability. Using computational fluid dynamics, the levels of mass transfer and shear stress in the system have been calculated and tuned to guide bioreactor design and operation in consideration of the tissues grown. The liver was chosen for preliminary testing of our novel device as it is a complex and highly perfused organ that should benefit from media perfusion and increased mass exchange[2].

Culture of HepG2-based liver models utilising Alvetex® Scaffold as a supporting membrane for the cells in this bioreactor system led to a more physiologically relevant morphology with greater expression of key drug transporters and formation of hepatocyte-specific structures such as bile canaliculi compared to static cultures. Further work with this system has incorporated additional oxygen control allowing the culture of ex vivo precision-cut liver slices which demonstrated sustained viability during multi-week culture. These results demonstrate the potential for this bioreactor technology to produce physiologically relevant cultures in a well characterised microenvironment whilst keeping technical complexity low.

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Abstract **#610**

EFFICIENT NON-VIRAL GENE MODIFICATION OF MESENCHYMAL STROMAL CELLS FROM UMBILICAL CORD WHARTON'S JELLY WITH POLYETHYLENIMINE

Ana Isabel Ramos-Murillo[P][1,2], Elizabeth Rodríguez[1], Karl Beltrán[2], Cristian Ricaurte[2], Bernardo Camacho[2], Gustavo Salguero[2], Rubén Darío Godoy-Silva[1]

[1] *Chemical and Biochemical Processes Research Group, Department of Chemical and Environmental Engineering, Faculty of Engineering, Universidad Nacional de Colombia, Bogotá D.C., Colombia*

[2] *Advanced Therapies Unit, Instituto Distrital de Ciencia, Biotecnología e Innovación en Salud (IDCBIS), Bogotá D.C., Colombia*

Corresponding author's email: airamosmu@unal.edu.co

Mesenchymal stromal cells (MSC) derived from human umbilical cord Wharton's jelly (WJ) have a wide therapeutic potential in cell therapy and tissue engineering because of their multipotential capacity, which can be reinforced through gene therapy in order to modulate specific responses. However, reported methodologies to transfect WJ-MSC using cationic polymers are scarce. Here, WJ-MSC were transfected using 25kDa branched-PEI and a DNA plasmid encoding GFP. PEI/plasmid complexes were characterized to establish the best transfection efficiencies with lowest toxicity. Expression of MSC-related cell surface markers was evaluated. Likewise, immunomodulatory activity and multipotential capacity of transfected WJ-MSC were assessed by CD2/CD3/CD28-activated PBMNC cocultures and osteogenic and adipogenic differentiation assays, respectively. An association between cell number, PEI and DNA content, and transfection efficiency was observed. The highest transfection efficiency (11.9±2.9%) at the lowest toxicity was achieved using 2 ng/uL DNA and 3.6 ng/uL PEI with 45,000 WJ-MSC in a 24 well-plate format (200 uL). Under these conditions, there was no significant difference between the expression of MSC-identity markers, inhibitory effect on CD3+ T lymphocytes proliferation and osteogenic/adipogenic differentiation ability of transfected WJ-MSC, as compared with non-transfected cells. These results suggest that functional properties of WJ-MSC were not altered after optimized transfection with PEI.

Abstract **#612**

TOPOLOGY OPTIMIZATION OF A MANDIBULAR RECONSTRUCTION PLATE AND BIOMECHANICAL VALIDATION

Carine Leung$_P$[1], David Koper[1,2], Lars Smeets[3], Paul Laeven[3], Gabriëlle Tuijthof[3], Peter Kessler[1]

[1] *Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Center, Maastricht, the Netherlands*

[2] *Department of Instructive Biomaterials Engineering and Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands*

[3] *Department of Instrument Design, Engineering and Evaluation, Maastricht University, Maastricht, the Netherlands*

Corresponding author's email: carine.leung@mumc.nl

Reconstruction plates, used to bridge segmental defects of the mandible, are subjected to repeated stresses of mastication. High stress concentrations can result in hardware failure (1). Topology optimization (TO) could reduce peak stress by computing the most optimal material distribution in a patient-specific implant (PSI) for mandibular reconstruction (2). The objective of this study was biomechanical validation of a TO-PSI. A computer-aided design (CAD) model of a mandible with a segmental defect was created and a standard-PSI was designed. A TO-PSI was then designed with a maximum stress equal to the ultimate tensile stress of Ti6Al4V (930 MPa) during a loading condition of 378 N. Finite element analysis (FEA) was used to analyze stresses during loading. Both designs were subsequently produced in triplicate from Ti6Al4V, fixated to polyurethane mandible models with segmental defects identical to the CAD model, and subjected to continuous compression. Fracture of the TO-PSI occurred at a median peak load of 334 N (range 304-336 N). Fracture of the mandible model in the standard-PSI group occurred at a median peak load of 1100 N (range 1010-1460 N). Failure locations of TO-PSI and standard-PSI samples corresponded to regions in the FEA where stresses exceeded the ultimate tensile strength of Ti6Al4V and polyurethane, respectively. Failure occurred within a 30% range of the predicted peak load, demonstrating a successful preliminary biomechanical validation of TO in the design process of mandibular reconstruction plates. Future work will focus on refining the finite element model, to further optimize the design process of TO-PSIs.

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Abstract **#613**

PERFUSION OF A BIOMIMETIC VASCULARIZED HYDROGEL DEVICE IN A NOVEL BIOREACTOR

Sanna Sämfors[P][1], Kristin Oskarsdotter[1], Claudia Villar Egea[1], Paul Gatenholm[1,2], Christian Bruderrek[3]

[1] *3D Bioprinting Center, Chalmers University of Technology, Sweden*

[2] *CELLHEAL AS, Norway*

[3] *Yuri AG, Germany*

Corresponding author's email: samfors@chalmers.se

We have designed a channeled device to facilitate supply of oxygen and nutrients throughout the engineered device in order to overcome the limitation in construct size due to oxygen diffusion. A channeled device mimicking a leaf was designed using CAD and the flow through the channels was optimized in simulation studies. The construct was fabricated by 3D bioprinting using Pluronic F-127 as sacrificial ink to print the channels. The actual leaf was bioprinted using tunicate cellulose nanofibers (TUNICELL) and alginate bioink and crosslinked in calcium chloride. The resulting device was a 35x18x3 mm leaf with one main channel connected to several side channels. Surface modification using periodate oxidation followed by laminin bioconjugation was performed to enhance cell adhesion. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis verified protein binding to the surface and Human umbilical vein endothelial cells (HUVECS) were used to show that endothelialization was possible. The device was perfused in a bioreactor developed under German Space Agency (DLR) contract for life science research missions under microgravity conditions. It was inherently developed for living cells and therefore is biocompatible and includes pumps and tanks to supply the cells with necessary nutrients as well as remove by-products of cellular metabolism. Media was successfully perfused through all the leaf hydrogel channels with periodic and continuous perfusion. The effect of flow rate on cell viability of stem cell laden hydrogel was evaluated. The biofabricated hydrogel leaf device shows great promise for the use as a perusable vascularized tissue engineered construct in various applications.

Abstract **#614**

ONGOING DEVELOPMENT OF A HUMAN 3-DIMENSIONAL TISSUE-ENGINEERED MODEL TO STUDY RIGHT HEART FAILURE

Aida Llucià-Valldeperas[P][1], Rowan Smal[1], Paul JM Wijnker[2], Jolanda van der Velden[2], Harm-Jan Bogaard[1], Anton Vonk-Noordegraaf[1], Marie-José Goumans[3], Frances S de Man[1]

[1] *Department of Pulmonology, Amsterdam UMC*

[2] *Department of Physiology, Amsterdam UMC*

[3] *Department of Cell and Chemical Biology, LUMC*

Corresponding author's email: aida.llucia@gmail.com

INTRODUCTION: Pulmonary arterial hypertension (PAH) patients ultimately die of right ventricular (RV) failure. To date there is no treatment because of no suitable pre-clinical models to study the RV.Therefore, we aim to develop a RV failure model to unravel the mechanisms that determine the fate of the RV by implementing the 3-dimensional engineered heart tissue (EHT) approach[1] with cardiomyocytes derived from PAH-patient induced pluripotent stem cells (iPSCs).

METHODS: iPSCs from PAH and healthy subjects were differentiated into cardiomyocytes (iPSC-CMs)[2], incorporated into the EHT, and cultured for 28 days (D). By changing stiffnes of the post we induced afterload [1]. Functional measurements (force, frequency, time and velocity) were achieved. Statistical analyses between normal and stiffer posts, and D14 or D28 were performed.

PRELIMINARY RESULTS:

We observed functional differences between control and PAH-EHTs with normal posts. After D14, PAH-EHTs generated higher force (P=0.001), contraction (P=0.013) and relaxation (P=0.002) times, and contraction (P=0.011) and relaxation (P=0.059) velocities compared to control EHTs. After D28, PAH-EHTs showed lower force (P=0.043), contraction (P=0.087) and relaxation (P=0.007) times, and contractile (*P=0.025) and relaxation (*P=0.038) velocities compared to control EHTs. There were no functional differences between normal and stiffer EHTs, except increased beating frequency (*P=0.017) on PAH-EHTs compared to control EHTs at D14, probably due to small group size (N=2).

CONCLUSIONS:

Under physiological conditions, control EHTs are more stable than PAH-EHTs, which force was drastically reduced at D28. Further optimization and profound characterization will be useful to unravel pathophysiological insights, as well as disease modeling and drug screening.

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Abstract **#615**

EFFICIENCY OF MINIMALLY MANIPULATED BONE MARROW CELLS TO REGENERATE OSTEOCHONDRAL DEFECTS

Chaozong Liu[P][1], Maryam Tamaddon[1]
[1] *University College London*
Corresponding author's email: chaozong.liu@ucl.ac.uk

Bone marrow concentrate (BMC) is increasingly used as biological tool for the orthopaedic applications and it serves as a source of growth factors and mesenchymal stem cells (MSCs) [1] in a one-step procedure. This study is to investigate the efficacy of BMC in conjunction with an osteochondral (OC) scaffold for treatment of osteochondral defects in sheep knee joints.

Bone marrow was aspirated from iliac crest of the sheep in group 2 and was processed using NTL Biologica kit in the operating theatre. An osteochondral defect was created in medial condyle, scaffold with or without BMC was implanted in the osteochondral defect. The tissue was retrieved 6 months post-op, and bone integration and cartilage formation were examined to assess the effect of BMC on cartilage regeneration.

Large variations (up to 2 times) were observed between the TNC of different sheep. ICRS scoring of regenerated cartilage showed no statistically significant differences between the BMC and non-BMC groups ($p>0.05$). Histological staining of cartilage with Alcian Blue and Safranin-O showed similar cartilage formation in both groups qualitatively and quantitatively (Bern Score), there was no significant difference between the cartilage quality of the groups. Bone regeneration (%BV/TV) was higher in the BMC-containing group (62.78%±7.9 vs 58.62% ±13), however the difference was not significant ($p>0.05$). No

Collectively, these data suggest that BMC seems to have limited effect on regeneration of bone and cartilage in osteochondral defects. Although bone regeneration was slightly improved the improvement was not statistically significant.

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Abstract **#616**

COUPLED IN-SILICO AND IN-VITRO MODELLING OF HEPATOCYTE CELL AGGREGATION AS A STEP TOWARDS IMPROVED BIOMATERIAL RISK ASSESSMENT

Tijana Sustersic[1,2,3], Milica Nikolic[2,3,4], Constantin-Edi Tanase[5], Amir Ghaemmaghami[5], Nihal Engin Vrana[6], Nenad Filipovic[1,2,3]

[1] *Faculty of Engineering, University of Kragujevac, Kragujevac, Serbia*

[2] *Steinbeis Advanced Risk Technologies Institute doo Kragujevac, Kragujevac, Serbia*

[3] *Bioengineering Research and Development Center, Kragujevac, Serbia*

[4] *Institute of Information Technologies, University of Kragujevac, Kragujevac, Serbia*

[5] *School of Life Sciences, Faculty of Medicine & Health Sciences, University of Nottingham, UK*

[6] *Spartha Medical, Strasbourg, France*

Corresponding author's email: tsustersic@risk-technologies.com

Introduction: The formation of spheroidal multicellular aggregates for hepatocytes has drawn attention as it has been shown to closely mimic the natural environment found in vivo [1]. Due to many factors that influence the formation of aggregates/spheroids, coupled in-silico and in-vitro modelling of hepatocyte cell aggregation is important in order to reduce the number of experiments and optimize the necessary conditions to form the aggregates/spheroids.

Experimental methods: Differentiated human hepatocytes (HepaRG) and hepatic stellate cells (LX-2) have been used to form hepatic spheroids at a ratio of 4:1 similar to that of parenchymal:nonparenchymal cells in the liver tissue. Formation of the hepatic spheroids was achieved using 2 conventional methodologies i.e. low adherent coating surface to form spheroids and a high-density array of pyramid-shaped microwells, into which a suspension of single cells is centrifuged-AggreWell system.

Numerical methods: Partial differential equations explain the early stages of the cell aggregation process, starting from the principles of mass and momentum balance for cells, culture medium, and ECM production [2]. Based on many input parameters including different material characteristics for the low adherent coating and cells etc., the model predicts the cell volume fraction, velocity of the cells, and cell density as a function of time.

Results and conclusions: Developed model is able to predict formation of aggregates and how different parameters affect the number of aggregates, as well as their speed of aggregation. Developed model can be used for experimental planning and is a step towards improved biomaterial risk assessment.

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Abstract **#617**

OPTIMIZATION OF 3D PRINTING NOZZLES FOR BIOENGINEERING APPLICATIONS VIA COMPUTATIONAL MODELING AND DEEP LEARNING

Mario Milazzo[P][1,2], Chi-Hua Yu[1,3], Vincent Fitzpatrick[4], Cesare Stefanini[2], Serena Danti[1,2,5], David L. Kaplan[4], Markus J. Buehler[1]

[1] *Laboratory for Atomistic and Molecular Mechanics (LAMM), Dept. of Civil and Environmental Engineering, Massachusetts Institute of Technology, United States of America*

[2] *The BioRobotics Institute, Scuola Superiore Sant'Anna, Italy*

[3] *Department of Engineering Science, National Cheng Kung University, Tainan, Taiwan*

[4] *Department of Biomedical Engineering, Tufts University, Medford, MA 02155, United States*

[5] *Dept. of Civil and Industrial Engineering, University of Pisa, Italy*

Corresponding author's email: milazzo@mit.edu

Additive manufacturing Technologies have changed the way we design and fabricate structural components, especially for bioengineering applications (e.g., prosthetic replacements, biocompatible tissues for in vitro studies) (1). We present an in silico analysis of a 3D printing nozzle, investigating the best sagittal cross section to minimize the shear stress on the ink and, thus, improving the printing quality. We employ a synergistic approach based on a finite-element model elaborated with Comsol Software that provides labeled data for a deep learning model, developed with Jupiter Notebook (2). We build an axysimmetric model of the nozzle with linear features, whose dimensions fall in the range of commercial components. While the dye is modeled as a rigid fixed component, we model the ink as a deformable compound consisting of silk/hydroxyapatite, a composite that is used to mimic bone (3). The deep learning model is trained by thousands of samples that include several key features for 3D printing (e.g., final diameter of the nozzle, volume flow rate). The results show a good ability to predict the shear stress of the nozzle (≈75%), and we further extend this model with an evolutionary algorithm to obtain an optimal design of the nozzle based on the specific ink. This methodology can potentially change the way we currently design 3D printing nozzles by adapting the structural topology of the nozzle to the specific ink and printing applications, especially in those fields in which strict design requirements play a key role on the final product.

Keywords

3D printing; Machine Learning; Topology Optimization

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Abstract **#618**

INJECTABLE HYPERBRANCHED POLY(B-AMINO ESTER) HYDROGELS WITH ON-DEMAND DEGRADATION PROFILES TO MATCH WOUND HEALING PROCESSES

Qian Xu[P][1], Sigen A[1], Wenxin Wang[1]

[1] *University College Dublin*

Corresponding author's email: qian.xu@ucdconnect.ie

Injectable hydrogels, with the ability to be introduced through minimal invasion and to fill any defect or cavity, have been extensively used for cell delivery in tissue regeneration in recent years. However, due to limited control of the material degradation process to match tissue integration, so far the developed injectable hydrogels have not fulfilled their potential.[1] Herein, an injectable hydrogel was developed with optimized degradation profiles to treat two different types of humanized excisional wounds (acute and diabetic).

Biodegradable hyperbranched poly(b-amino ester)s (HP-PBAEs) were newly designed and synthesized via "A2 + B4" Michael addition polymerization, and displayed fast gelation with thiolated hyaluronic acid (HA-SH) via a "click" thiol–ene reaction. Rheological measurements, degradation and water retention capacity tests, and dynamic/strain sweep tests were conducted on a series of HP-PBAE/HA-SH hydrogels.

The injectable hydrogels showed controllable and adjustable degradation profiles both in vitro and in vivo, using diamines with different alkyl chain lengths and poly(ethylene glycol) diacrylates with varied PEG spacers.

Two hydrogels with optimized degradation rates were used to encapsulate adipose-derived stem cells (ADSCs) and injected onto humanized excisional wounds (the humanized wound model can better mimic the human cutaneous healing process). Hydrogels with a faster degradation rate exhibited better wound healing in non-diabetic wounds, while hydrogels with a slower degradation rate showed a faster wound closure rate and significantly promoted the healing process as evidenced by histological assays for diabetic wounds.

The new HP-PBAE-based hydrogels in combination with ADSCs provide an alternative approach in accelerating the wound healing process.

Keywords

Injectable hydrogel; Hyperbranched polymers; Diabetic wound healing

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Abstract **#621**

IN SITU GELLING POLYSACCHARIDE-BASED, DOUBLE NETWORK HYDROGEL FOR CARTILAGE REGENERATION THERAPIES

Marko Mihajlovic$_P$[1,2], Margot Rikkers[3], Blessing Ilochonwu[1], Keita Ito[2,3], Tina Vermonden[1]

[1] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands*

[2] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands*

[3] *Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands*

Corresponding author's email: m.mihajlovic1@uu.nl

In osteoarthritis, there is progressive damage of cartilage tissue. Because of the limited self-healing capacity of cartilage, it is important to investigate regenerative therapies. With their high water content, hydrogels are suitable for regenerative medicine applications. Chondroitin sulfate (CS) and hyaluronic acid (HA) are glycosaminoglycans found in native cartilage tissue. Therefore, hydrogels based on CS and HA are potentially useful for cartilage replacement therapies. However, such hydrogels usually lack mechanical properties when compared to synthetic hydrogels. Double network (DN) hydrogels are a class of hydrogel materials based on two interpenetrated networks, which often deliver promising mechanical properties. Here, we aimed to develop an in situ gelling, double network hydrogel, made out of CS and HA. The crosslinking chemistries were based on dynamic covalent interactions (Diels-Alder and hydrazone bonds). Two types of functionalized CS were prepared: a CS containing aldehyde groups (degree of functionalization DoF 10%), and a CS bearing maleimide moieties (DoF 15%). HA was functionalized with methylfuran groups (DoF 40%). In addition, adipic dihydrazide was used to crosslink oxidized CS. Gelation of the single networks was successful. These polymers were further combined to form a DN hydrogel (at 20 wt%), characterized by tunable viscoelastic and stress relaxation properties. It was shown that hydrazone-based network was shear-thinning, reversible and self-healing, which allowed for initial processing (e.g. extrusion), before the system was further crosslinked by Diels-Alder reaction. Cytocompatibility of the DN hydrogel is being investigated in order to assess suitability of the system to carry cells responsible for cartilage regeneration.

Keywords

polysaccharides; double network; osteoarthritis

Abstract **#622**

OSTEOCHONDRAL SCAFFOLD INNOVATION FOR EARLY INTERVENTION OF CARTILAGE DEFECT: FROM BENCH TO CLINIC

Chaozong Liu[P][1], Maryam Tamaddon[1]
[1] *University College London*
Corresponding author's email: chaozong.liu@ucl.ac.uk

The treatment of cartilage defects remains a challenge because treatments to date have failed to achieve a complete restoration of the joint cartilage surface and its properties. We have invented a novel osteochondral scaffold based on Titanium and PLGA matrix reinforced collagen/hydroxyapatite composite system for repair of large osteochondral defects, evaluated the in vivo performance using sheep condyle model, and has proceeded to first in man clinical study [1, 2].

Sheep medial formal condyle mode was used to evaluate the in vivo performance. The tissues were retrieved six months post-operation. The retrieved tissues were examined by histological and histomorphometry in terms of mechanical stability and biological fixation of the scaffold. A clinical dog shoulder model was used for preclinical study of the safety and efficacy of the osteochondral scaffold.

Retrieved tissue, from sheep condyle model, examinations revealed that good levels of defect fill, and formed hyaline cartilage-like tissue, and integrated well with the surrounding healthy tissues. Immunohistochemical staining revealed formation. pQCT and micro-CT examinations revealed bone ingrowth into Titanium matrix and form a strong integration with the surrounding bone. The bone-implant contact was calculated to be 61% (±15), and BV/TV ratio was significantly higher (p=0.01) in the scaffold group (~70%) compared to control group (~45%).

The sheep study has demonstrated the developed scaffold has the strength needed to bear the physical load of the joints and its patented biomedical structure encourages consistent cartilage fill. This novel scaffold would enable surgeons to intervene earlier, to prevent progression of the condition.

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Abstract **#623**

PRECLINICAL EFFICACY AND SAFETY EVALUATION OF A BIOARTIFICIAL KIDNEY DEVICE

Milos Mihajlovic$_P$[1], Merle Krebber[2], Koen Westphal[1], Silvia Mihaila[1], Rolf Sparidans[1], Danilo Donato[3], Karin Gerritsen[2], Markus Storr[3], Rosalinde Masereeuw[1]

[1] *Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands*

[2] *Department of Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, The Netherlands*

[3] *Baxter International, Research and Development, Hechingen, Germany*

Corresponding author's email: m.mihajlovic@uu.nl

Aim: Many patients affected by end stage kidney disease depend on dialysis for survival. However, hemodialysis does not remove efficiently protein-bound uremic toxins (PBUTs), thus leading to severe comorbidities. Therefore, novel treatments, such as a bioartificial kidney (BAK), are needed. Here, efficacy and safety of a BAK device based on conditionally immortalized proximal tubule epithelial cells (ciPTECs) and hollow fiber membranes (HFM), are being evaluated.
Methods: Firstly, ciPTECs compatibility with PES/PVP HFM was determined. Subsequently, a prototype BAK was built to evaluate its capacity to remove PBUTs in vivo. Finally, stability of membrane coating was assessed by LC-MS/MS quantification.
Results: Collagen IV, L-DOPA [1,2] and the combination of these coatings were found to support cell attachment and formation of epithelial monolayers (F-actin staining). The polarization and tightness of monolayers were confirmed by increased expression of ZO-1, cilia presence and reduced FITC-inulin leakage by 70.7% ($p<0.0001$). The activity of OAT-1 transporter was determined by means of fluorescein uptake in absence and presence of probenecid (100±15.7% vs 64.9±15.1%; $p=0.18$). L-DOPA was found to be released from coated HFM only during the first 3 days (6.7±1.7 ng/ml, $p<0.005$). For in vivo efficacy studies, prototype BAK devices containing 10 HFM with surface area of 19.1 cm2 and blood and dialysate compartment volumes of 0.12 ml and 12 ml respectively, were prepared.
Conclusion: CiPTECs can be grown on L-DOPA coated PES/PVP HFM, to form functional epithelial monolayers. Prototype BAK device was successfully built to determine PBUTs clearance in rodents, and the results are expected soon.

Keywords

bioartificial kidney; efficacy; prototype

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Abstract **#624**

ENZYMATIC DIRECT ON-CELL CROSSLINKING (DOCKING) ENABLES MECHANOTRANSDUCTION IN NON-ADHERENT MATERIALS

Tom Kamperman[P][1,2], Sieger Henke[1], João Crispim[1], Pieter J. Dijkstra[1], Wooje Lee[3], Herman Offerhaus[3], Martin Neubauer[4], Andreas Fery[5], Alexandra M. Smink[6], Bart de Haan[6], Su Ryon Shin[2], Marcel Karperien[1], Jeroen Leijten[1]

[1] *Department of Developmental BioEngineering, Faculty of Science and Technology, Technical Medical Centre, University of Twente, The Netherlands*

[2] *Division of Engineering in Medicine, Brigham and Women's Hospital, Harvard Medical School, USA*

[3] *Optical Sciences, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands*

[4] *Physical Chemistry II, University of Bayreuth, Germany*

[5] *Leibniz-Institut für Polymerforschung Dresden e.V. and Technische Universität Dresden, Germany*

[6] *Department of Pathology and Medical Biology, Section of Immunoendocrinology, University Medical Center Groningen, University of Groningen, The Netherlands*

Corresponding author's email: t.kamperman@utwente.nl

Cells dynamically interact with their microenvironment via cell-matrix interactions. However, engineered biomaterials are often endowed with permanent and static adhesive moieties such as RGD motives. These adhesion strategies are associated with increased chronic inflammation upon implantation [1]. Here, we pioneer a novel cell binding technology, called 'Direct On-cell Crosslinking' (DOCKING), that directly tethers intrinsically non-adherent (e.g., RGD-free) biomaterials to cells via a universal and cytocompatible enzymatic crosslinking reaction between polymers and cellular proteins.

Specifically, we explored crosslinking of tyramine-modified biomaterials such as dextran (Dex-TA) to tyrosine residues on mesenchymal stem cells (MSCs). Subcutaneous implantation of Dex-TA hydrogel disks in C57BL/6 mice revealed significantly less fibrotic capsule formation as compared to Dex-TA-RGD. Adhesion of individually encapsulated cells within Dex-TA microgels via DOCKING was visualized by confocal and scanning electron microscopy. Mass spectrometry confirmed that DOCKING-targeted proteins associated with the cellular adhesome [2] and particularly connected to integrins alpha-5 and beta-1. Dynamically tuning the stiffness of cell-laden microgels via in situ enzymatic post-curing confirmed transduction of mechanical cues from the biomaterial to the encapsulated MSCs. For example, mechanotransduction as a direct result of DOCKING was indicated by upregulation of adipogenic and osteogenic genes as early as on day 1 of differentiation in soft (2.5 kPa) and stiff (45 kPa) microgels, respectively, and was likely mediated through nuclear lamin A/C.

In conclusion, enzymatic DOCKING provides a novel strategy that minimizes adverse host responses such as fibrous capsule formation to biomaterials, while enabling mechanical interactions with encapsulated cells to steer cell fate via mechanotransduction.

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Abstract **#626**

BIOLOGICALLY-ENGINEERED PEDIATRIC TRI-TUBE VALVED CONDUIT EVALUATED 52 WEEKS IN THE GROWING LAMB

Robert Tranquillo$_P$[1], Sandra Johnson[1], Matthew Lahti[1], James Berry[1], John Carney[1], Jirong Li[1], Ryan Hill[2], Kirk Hansen[2], Greeshma Nair[1], Zeeshan Syedain[1]

[1] *University of Minnesota*

[2] *University of Colorado – Anschutz Medical Campus*

Corresponding author's email: tranquillo@umn.edu

There is a dire need for a heart valve that grows with children. We fabricated tubes of biologically-engineered matrix previously shown to regenerate and grow as a pulmonary artery replacement in lambs, and implemented a novel valve design made from sewing three of these tubes together with degradable suture that confers growth potential. Seven lambs were implanted with tri-tube valved conduits in sequential cohorts and compared to bioprosthetic conduits. Valves implanted in two lambs of the first cohort of four animals functioned with only mild regurgitation and systolic pressure drops <10 mmHg up to 52 weeks post-implantation, during which the valve diameter increased from 19 mm to a normal ~25 mm. In a second cohort of three animals, an additional tube was used to create a sleeve around the tri-tube valve to counteract faster root growth relative to the leaflets. Two valves exhibited only trivial-to-mild regurgitation at 52-weeks with similar diameter increase and systolic pressure drops of < 5 mm Hg. The third animal exhibited moderate regurgitation at 52 weeks correlating to hyper-increase of valve diameter. In all explanted valves, the leaflets remained thin and pliable with only sparse, punctate microcalcification at most, and they contained interstitial cells and an endothelium progressing from the base of the leaflets. These results are superior in terms of calcification and hemodynamic function compared to reported studies for clinically-used pediatric bioprosthetic valves tested in the same model. The results demonstrate the potential for long-term valve growth of this “off-the-shelf” tri-tube valved conduit in children.

Abstract **#627**

PSEUDOPOLYROTAXANE HYDROGELS FOR NEURAL TISSUE ENGINEERING APPLICATIONS

Emmanuel Kanelos$_P$[1], Rylie Green[1], Josef Goding[2]

[1] *Imperial College London*

[2] *Imperial College london*

Corresponding author's email: edk16@ic.ac.uk

Hydrogels are a cornerstone of neural tissue engineering, replicating extracellular matrix and supporting cell growth. An underexplored class of hydrogels are pseudopolyrotaxanes (PPRs) which possess a bead on a string supramolecular structure such as that formed between polyethylene-glycol (PEG) and cyclodextrin (CD). These systems possess desirable properties such as shear-thinning, easy biofunctionalization and tuneable mechanics for tailorable systems. Previous studies have not achieved degradability, used single biofunctionalisations and focused only on bone engineering. Here swelling, degradation and mechanics of PEGDA/CD gels are explored. Finally, we investigated the effect of dithiothreitol (DTT) on hydrogel degradation.

In PEGDA-CD rotaxane gels it is observable that swelling is decreased significantly with the addition of CD, due to the hydrophobic complex formed. Apart from initial sol-fractions, mass-loss does not occur over 28 days regardless of CD loading. Likewise, compression studies established the Young's modulus between 4-6 kPa and unaffected by CD loading. To address degradation, dithiothreitol (DTT) was introduced at molar ratios of 1:4,1:10, and 1:20 (DTT:PEG). A dose dependent response was observed, the highest concentration resulted in 75% mass-loss, whereas the lowest resulted in none. Thus, DTT is a low-cost method to control degradation. This is the first instance of thio-ether acrylate rotaxanes being investigated for neural tissue engineering.

Thio-ether PEGDA/CD gels are versatile systems with easy introduction of multiple functionalisations and tailorable mechanics. Coupled with shear-thinning properties there is great potential in injectable brain tissue engineering applications. Future work will investigate the biofunctionalization of CD for neural applications and full characterisation of mechanics.

Abstract **#628**

THE EFFECT OF SERUM DEPRIVATION ON OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS (ASCS) ON A 3D SILK FIBROIN SCAFFOLD

Gerrit Freund[P][1], Tim Ruhl[1], Lara Lingens[1], Benedikt Schäfer[1], Aleksander Drinic[2], Marius Köpf[2], Justus P. Beier[1], Rebekka Götzl[1]

[1] *Department of Plastic Surgery, Hand and Burn Surgery, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen*

[2] *Fibrothelium GmbH, c/o TRIWO Technopark Aachen, Philipsstr. 8, 52068 Aachen*

Corresponding author's email: gfreund@ukaachen.de

Issue: Stem cells obtained from human fat tissue (ASCs) are multipotent progenitor cells, being able to differentiate into different mature cells, including osteocytes. Standard cultivation with fetal bovine serum (FBS) carries risks such as immunization and xenogeneic infection. Replacement of FBS is inevitable for future translation into clinics. However, altered media compositions could influence ASC proliferation and differentiation. In this study, ASCs were expanded and differentiated at decreasing concentrations of FBS on a 3D bromid free fibroin sponge to evaluate the effect on cellular vitality as well as on osteogenic differentiation.

Methods: We examined proliferation using PrestoBlue conversion (PB) and differentiation into the osteogenic lineage over a period of 14 days with 0-2% FBS concentration. Alkaline phosphatase activity (ALP), calcium deposition measured by cresolphthalein staining (CP) and the osteocalcin concentration through ELISA were taken for quantification of osteogenesis.

Results: The CP labelling, ALP activity and osteocalcin concentration increased with rising FBS concentration. However, osteogenic differentiation was also achieved at 0% FBS and showed significantly higher CP values in comparison to the control group. Osteogenic differentiation was still obtained on declining proliferation. Overall elevated measurements for ALP activity, cell numbers and osteocalcin secretion were observed for the 3D in comparison to the 2D culture.

Conclusion: Based on this data, it seems possible to achieve osteogenic differentiation of ASCs independently from xenogeneic serum supplements in a 3D culture by using silk fibroin scaffolds. This would lead to a reduction of xenogenic risks with a prospective in vivo and possible clinical translation.

Keywords

Adipose Stem Cells; Bone Tissue Engineering; Silk Fibroin

Abstract **#629**

TISSUE-ENGINEERED TRANSCATHETER VEIN VALVE

Robert Tranquillo[1], Anders Jenson[1], Puja Patel[1], Cole Feagler[1], Logan Bahmer[1], Rumi Faizer[1], Zeeshan Syedain[P][1]

[1] University of Minnesota

Corresponding author's email: syeda004@umn.edu

Chronic venous insufficiency affects over 2 million patients in the US alone, with severe cases involving thousands of patients with chronic leg ulcers and potential amputation. A transcatheter vein valve made from a biologically-engineered matrix possessing the ability to regenerate has the potential to provide both valve function and long-term hemocompatibility and durability because the matrix becomes endothelialized and populated with host tissue cells. We developed a novel tissue-engineered transcatheter vein valve (TEVV) on a Nitinol stent1 and demonstrated function and durability in vitro. Tissue was grown from fibroblasts in fibrin gel so as to embed the stent, with a tubular extension of the engineered tissue from one end of the stent that was stitched along opposite sides and everted into the stent to form a bileaflet valve. Following decellularization, to create an “off-the-shelf” TEVV comprised of the resulting collagenous matrix, it was tested in a pulse duplicator to evaluate hydrodynamic properties. The TEVV was shown to have forward pressure drops in the range of 2-4 mmHg, low closing volume, and nil regurgitation. Further hydrodynamic tests were performed after crimping and then again after 1 million cycle durability testing, showing no degradation of valve performance or any visual damage to the matrix. The TEVV held over 600 mmHg backpressure after the durability testing, ensuring the valve would withstand pressure spikes well outside of the normal in vivo range. Catheter-based delivery into the ovine iliac vein demonstrated TEVV closing 2 weeks p.o. and endothelialization without thrombosis 8 weeks p.o.

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Abstract **#630**

BIOLOGICALLY-ENGINEERED MECHANICAL MODEL OF A CALCIFIED ARTERY

Robert Tranquillo$_P$[1], Sandra Johnson[1], Zeeshan Syedain[1], Ryan Hill[2], Kirk Hansen[2], Han Seung Lee[1], Greeshma Thrivikraman[1]

[1] *University of Minnesota*

[2] *University of Colorado-Denver*

Corresponding author's email: tranquillo@umn.edu

Vascular calcification is a commonly occurring pathological process and is recognized as an independent prognostic marker for cardiovascular morbidity and mortality. Recent progress in developing novel therapies to modify vascular calcification is critically hampered due to the lack of reliable in vitro experimental models that recapitulate the structural and mechanical attributes of calcified arteries. In this study, we show the ability to model the behavior of diffuse vascular calcification in vitro using biologically-engineered grafts approximating the composition, structure, and mechanical properties of arteries.1 Transmural calcification was achieved by exposing the acellular grafts of collagenous ECM to complete medium containing elevated Calcium (Ca) and Phosphate (P) concentrations. It was found that increasing the serum concentration from 2% to 10% increased the extent and degree of calcification based on histochemical, ultrastructural, chemical and thermal analyses. The presence of variably-sized spherical calcific deposits within the matrix further confirmed its morphological similarity to pathologic calcification. Mechanical testing demonstrated up to a 16-fold decrease in compliance due to the calcification, consistent with prior reports for calcified arteries. The model developed thus has potential to improve the design and development of interventional devices and therapies for the diagnosis and treatment of arterial calcification.

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Abstract **#631**

MODULATION OF MYOGENIC CELL FRACTION AND MYOTUBE FORMATION OF ISOLATED MYOGENIC PROGENITORS CELLS THROUGH COATING OF THE CELL CULTURE SURFACE

Lisanne Terrie[P][1], Mathieu Vandamme[1], Melanie Gerard[1], Lieven Thorrez[1]

[1] *Tissue Engineering Lab, Dep. Development and Regeneration, KU Leuven Kulak*

Corresponding author's email: lisanne.terrie@kuleuven.be

Skeletal muscle tissue engineering has several applications such as the replacement of muscle tissue after volumetric muscle loss, the use as a 3D in vitro model system to study muscle physiology and in vitro drug testing. Selecting and expanding the proper cells as the starting source is an important factor to guarantee that engineered muscle closely mimics the in vivo situation. Satellite cell-derived myoblasts are often used because satellite cells are the native adult stem cell precursors for skeletal muscle. Obtaining these myoblasts in sufficient high numbers and with a high purity without compromising their myogenic potential is a challenge. In this work we tested specific coatings used to culture the myoblasts in vitro after harvesting the cells from human muscle biopsies. We monitored cell yield, cell morphology, myoblast percentage and myogenic differentiation capacity. Five different coatings were evaluated: collagen, gelatin, laminin, poly-L-ornithin/laminin and poly-L-lysine. The laminin and poly-L-ornithin/laminin coating resulted in improved myoblast attachment and proliferation compared to the other coatings. The percentage of myoblasts in the cell pool was evaluated with a desmin staining and the ability of the myoblasts to fuse into myotubes was assessed with a tropomyosin staining. The laminin-based coatings were found to maximize the myogenic versus non-myogenic ratio and resulted in the highest number of multinucleated myotubes. We conclude that the laminin-based coatings were best suited for expansion and support of myogenic capacity of satellite cell-derived myoblasts.

Abstract **#632**

DESIGN BY NATURE: CREATING A TISSUE-SPECIFIC HYDROGEL FROM DECELLULARIZED HUMAN LIVERS FOR IMPROVED EXPANSION AND DIFFERENTIATION OF LGR5+ HEPATOBILIARY ORGANOIDS

Jorke Willemsep[1], Jeroen de Jonge[1], Diana van der Ven[1], Eline V.A. van Hengel[1], Bart Spee[2], Luc J.W. Van der Laan[1], Monique M.A. Verstegen[1]

[1] *Department of Surgery, Erasmus MC-University Medical Center, Rotterdam, the Netherlands.*

[2] *Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine Utrecht University, Utrecht, the Netherlands*

Corresponding author's email: j.willemse@erasmusmc.nl

Human hepatobiliary LGR5+ organoids have great potential for tissue engineering purposes, as they can potentially regenerate damaged hepatobiliary tissue. However, the use of non-clinical grade mouse tumor extracellular matrix (ECM) extracts, such as Matrigel, is hampering clinical applications of these organoids. Furthermore, components of Matrigel hamper the differentiation towards mature cholangiocytes or hepatocytes. The aim of this study is to create clinical-grade tissue-specific hydrogels from human liver ECM for improving differentiation of hepatobiliary organoids.

Human livers (deemed unsuitable for transplantation, N=5) were fully decellularized in a pressure-controlled perfusion device using 4% Triton-X-100 + 1% ammonia. ECM components were extracted by pepsin digestion of the ECM to gain a viscous liver-based hydrogel. Subsequently, human liver organoids (N=15) were initiated in this hydrogel. Matrigel was used as control. Purpose-designed spinner flask were used to explore the large-scale expansion of organoids in liver ECM-hydrogel.

Liver-derived ECM hydrogels support initiation and proliferation of hepatobiliary organoids similar to BME controls. Gene expression of albumin was increased 142-fold, whereas expression of KRT-7 and KRT-19 decreased 2-fold in liver ECM hydrogel, indicating improved differentiation towards hepatocytes. Spinner flask cultures yielded up to 6-fold increase in cells/ml compared to static culture conditions.

This study shows the feasibility of creating liver-specific hydrogels, which supports differentiation of human hepatobiliary organoids. The spinner flasks were effective and allow for large-scale clinically relevant production of cells. The liver ECM hydrogels can provide an important step forward in applying these organoids in clinical settings for regenerative medicine purposes.

Keywords

Organoids; Decellularized liver; Hepatobiliary

Abstract **#633**

PHOTO-CROSSLINKABLE GELATIN-BASED HYDROGELS FOR ADIPOSE TISSUE ENGINEERING

Lana Van Damme$_P$[1]

[1] *University of Ghent*

Corresponding author's email: lana.vandamme@ugent.be

There exists a clear clinical need for adipose tissue reconstruction strategies to repair soft tissue defects which outperform the currently available approaches. In this respect, additive manufacturing has shown to be a promising alternative for the development of larger constructs able to support adipose tissue engineering. In the present work, crosslinkable hydrogels were developed which allowed cell encapsulation of adipose tissue-derived stem cells to be applied in extrusion-based bioprinting. To this end, norbornene-functionalized gelatin (Gel-NB) was combined with thiolated gelatin (Gel-SH) [1] enabling a thiol-ene click chemistry to develop a superior, cell compatible extracellular matrix (ECM) mimic.
First, the materials were synthesized and 2D sheets were characterized physico-chemically by performing gel fraction, swelling, mechanical and in vitro biodegradability assays while using conventional methacrylated gelatin (Gel-MA) [2] as benchmark. Next, both biocompatibility and the differentiation potential of encapsulated adipose tissue-derived stem cells (ASCs) were analysed via live/dead staining as well as Bodipy/DAPI and a triglyceride assay. In vivo experiments are currently ongoing assessing the differentiation potential and neovascularisation via ex vivo histology.
The results showed that the materials are able to mimic the physico-chemical characteristics of the natural ECM of native adipose tissue (viability >70%). Hence, the hydrogels could be of great interest towards future development of adipose tissue constructs and tissue engineering in general as it could offer a shift towards a patient-specific, 3D-printed, reconstructive approach, that can potentially be safer and more cost-effective compared to current clinical approaches.

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Abstract **#634**

SHIKONIN-LOADED BIOPOLYMERIC NANOCARRIERS FOR MACROPHAGE TARGETING AND INFLAMMASOME MODULATION

Matias Cardoso[1,2], Vitor Gaspar[1], Ricardo Silvestre[2], Iola M Duarte[1], João F Mano[P1]

[1] *CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal*

[2] *ICVS/3B's – PT Government Associate Laboratory, Braga, Portugal*

Corresponding author's email: matias.cardoso@ua.pt

Host immune response to medical devices and implantable biomaterials is key to their functional integration. Several cell types, proteins and extracellular components are involved in this innate response. Macrophages are essential in the regulation of the inflammasome and, thus, in the overall innate response, leading either to inflammation resolution or persistence. The development of biomaterial-based platforms to modulate the inflammasome and achieve favorable inflammatory responses is a promising strategy towards the promotion of implant integration and tissue regeneration. In the present work, we have developed macrophage-targeted biopolymeric-based nanocarriers for the controlled release of immunomodulatory metabolic drugs. Shikonin, a natural naphthoquinone with anti-inflammatory and inflammasome-attenuating properties, was encapsulated into zein-hyaluronic acid nanoparticles. These nanocarriers had hydrodynamic diameters near 200 nm and were efficiently taken up by human macrophages, showing no toxicity up to 1 mg/mL. The ability to modulate the cells inflammasome was also demonstrated through assessment of caspase-1 activity and IL-1β production. Moreover, NMR metabolomics was employed to monitor changes in the extracellular medium composition upon inflammasome activation and attenuation. This approach provided novel insight into how metabolites and metabolic pathways influence macrophage responses to biomaterials and immunomodulatory small molecules. Looking forward, this work is expected to foster the development of a novel class of versatile and cost-effective inflammasome-modulating biomaterials to mitigate implant-related chronic inflammation and promote tissue regeneration.

Keywords

Inflammasome; Nanoparticles; Immunomodulation

Abstract **#635**

BIOMATERIAL DATA MINING: A COMPARATIVE STUDY OF METHODS ON POLYDIOXANONE LITERATURE

Carla V. Fuenteslópezp[1], Austin McKitrick[2], Javier Corvi[3], Maria-Pau Ginebra[2], Osnat Hakimi[2,3]

[1] *Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, OX3 7DQ, UK*

[2] *Department of Material Science and Engineering, Universitat Politècnica de Catalunya*

[3] *Barcelona Supercomputing Center (BSC)*

Corresponding author's email: carla.fuenteslopez@eng.ox.ac.uk

Information extraction is fundamental to scientific research, however, when done manually, it's extremely time-consuming and often comprises re-work. Alternatively, automated data extraction renders up-to-date, quick, and easy access to information. Nevertheless, there is little precedence of using automated text mining tools to extract information in the biomaterials domain[1].

Here, we compare the ability of contemporary text mining tools to extract useful information from biomaterials abstracts. As a topic, we selected 'polydioxanone' which, despite being a widely-used biomedical material, has a relatively limited literature, thus enabling manual validation of results.

We filtered abstracts using a machine learning classifier, utilising a Support Vector Machine algorithm trained on a previously-published gold standard set[2]. Following corpus definition and validation, we compared information extraction tools, including PubTator, text analysis methods, and DEBBIE, an open-access annotation pipeline of biomaterials, implants and medical devices.Results were analysed in conjunction with a manual review of the literature and existing systematic reviews.

Our findings show that lexical annotations of diseases and species by non-specific tools were accurate, but there were some inaccurate tags and categories. DEBBIE, as a biomaterial-specific tool, can annotate several domain-specific categories missing in general text mining tools. The unsupervised learner provided a quick, but computationally-expensive manner of clustering abstracts into topics.

We noted that indexing polymer-related literature is challenging, as naming is heterogeneous, and abbreviations are extremely versatile. Also, most of the tools required some degree of user proficiency, limiting their use. As such, we recognise a need for field-specific and user-friendly tools, such as DEBBIE.

Keywords

Text Mining; Polydioxanone; Information Extraction

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Abstract **#636**

THE MODIFIED HEDGEHOG TECHNIQUE TO REATTACH CHONDRAL FRAGMENTS IN THE YOUNG ADULT KNEE – FOLLOW-UP WITH 7.0T MRI AT 3 MONTHS AND 1 YEAR AFTER SURGERY

Marloes Peters[P][1], Ralph Jeuken[1], Esther Steijvers[2], Wiel WIjnen[1], Daan Loeffen[1], Pieter Emans[1]

[1] *Maastricht University Medical Center, the Netherlands*

[2] *Ultra-High Field Centre Scannexus, the Netherlands*

Corresponding author's email: marloes.peters@maastrichtuniversity.nl

The modified “Hedgehog” technique is a new surgical technique to reattach pure chondral fragments. The calcified side of chondral fragments is multiply incised and trimmed obliquely for an interlocking fit in the defect site. The technique was applied to young adults in this preliminary report, using high-field Magnetic Resonance Imaging (MRI) as outcome measure.

Three patients with a femoral cartilage defect and concomitant pure chondral corpus liberum were operatively treated by the modified Hedgehog technique. Age at surgery ranged from 20.6–21.2 years, defect size ranged from 3.8–6.0 cm2. Patients were evaluated at three and twelve months after surgery by 7.0T MRI, including a proton density weighted sequence to assess morphological tissue structure and gagCEST imaging, T2-mapping to measure the biochemical tissue composition in terms of glycosaminoglycans (GAG) and collagen structure respectively.

Twelve months after surgery patients reported no pain and showed full range-of-motion.
Over time, morphological MRI visualized improvements in integration of the cartilage fragment with the surrounding cartilage, which was supported by biochemical MRI showing increased GAG values and decreased T2-mapping values at the defect edges.

The modified Hedgehog technique has advantages over earlier used methods to reattach chondral fragments that use metalwork or absorbable biomaterials and involve both biomaterial-related drawbacks. Although the number of subjects in the present study was small, the preliminary results show potential. High-field MRI has the ability to follow-up the quality of reattached piece of cartilage itself as well as the integration of the edges of the defect with the surrounding cartilage.

Keywords

cartilage defect; MRI

Abstract **#637**

THE MATRIX RELOADED: CREATING FUNCTIONAL HEPATIC CONSTRUCTS USING DECELLULARIZED HUMAN LIVER AND INTRAHEPATIC CHOLANGIOCYTE ORGANOIDS

Jorke Willemsep[1], Eric Safai[1], Luc J.W. van der Laan[1], Jeroen de Jonge[1], Monique M.A. Verstegen[1]
[1] *Department of Surgery, Erasmus MC-University Medical Center, Rotterdam, the Netherlands.*
Corresponding author's email: j.willemse@erasmusmc.nl

Liver transplantation is the only durable treatment for end-stage liver disease, but shortage of donor organs remains a limiting factor. Tissue engineered functional liver tissue could overcome this problem. LGR5+ intrahepatic cholangiocyte organoids (ICO), initiated from liver biopsies are an interesting cell source for hepatobiliary tissue engineering purposes. The aim of our study is to combine organoid technology with decellularization and recellularization techniques to make functional hepatic constructs.
Human livers (deemed unsuitable for transplantation, N=3) were decellularized using a pressure-controlled perfusion device with 4% Triton-X-100 + 1% ammonia. Circular sections were created using a dermal biopsy punch (Ø:8mm) and a cryotome (thickness:200µm). ICO were initiated from liver biopsies and added to the segments as a single cell suspension (25·104 cells/segment), which were cultured for up to 21-days (static conditions) or 7-days (microfluidic setup).
Under static conditions, organoids, incubated as single cell suspension, encapsulated the ECM, but did not infiltrate it. Phenotypically, these cells appeared to be columnar in shape, resembling cholangiocytes. Infiltration of ECM was achieved under flow conditions using a microfluidic setup. Gene expression and protein analysis revealed upregulation of hepatic (Albumin) and cholangiocyte (KRT-7) markers and downregulation of stem cell markers (LGR5) when compared to controls.
In conclusion, this study demonstrates that repopulation of decellularized human liver ECM with ICO is feasible and that these organoids exert self-organization and differentiation upon contact with the ECM. These initial results encourage further large-scale perfusion-based recellularization experiments in an effort to engineer functional and clinically relevant hepatic tissue in vitro.

Keywords
Hepatobiliary Organoids; Microfluidics; Transplantation

Abstract **#638**

DEVELOPING LIPID-BASED NANOPARTICLES FOR SYSTEMIC DELIVERY OF MESENCHYMAL STEM CELLS SECRETOME IN PARKINSON'S DISEASE

Sandra Barata-Antunes[P][1], Rui A. Sousa[2], Bruno F.B. Silva[3], António J. Salgado[1]

[1] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal and ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal

[2] Stemmatters, Biotecnologia e Medicina Regenerativa SA, Guimarães, Portugal

[3] INL - International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga s/n., Braga 4715-330, Portugal

Corresponding author's email: id7405@alunos.uminho.pt

Parkinson's disease (PD) is a neurodegenerative disease clinically characterized by motor disabilities. Current therapies are not being fully effective. Remarkably, the neuroregulatory molecules secreted by mesenchymal stem cells have been suggested as an alternative therapy. However, direct injection into the brain is the delivery approach that has been used in pre-clinical models [1]. Thus, the main goal of this work is to develop brain-targeting nanoparticles to deliver the secretome of MSCs and allow a systemic delivery treatment. For that, lipid-based nanoparticles were synthetized using different lipids and assembly approaches. The lipidic mesophase structures formed by mixing the lipid(s) with MSCs' secretome at different mass ratios were analysed by small-angle X-ray scattering (SAXS). The liquid crystalline phases were mechanically dispersed to produce nanoparticles. The particle size distribution and polydispersity index (PDI) of the final dispersions were evaluated by Dynamic Light Scattering (DLS). To determine the effect of the nanoparticles on cell viability, the MTS assay was performed using SH-SY5Y cells. SAXS analysis showed the formation of different liquid crystalline phases, depending on the lipid formulation used. Notably, these structures, which are promising self-assembled lipid systems for controlled release of drugs showed a high secretome ecapsulation efficiency. The DLS results showed hydrodynamic diameters smaller than 200 nanometers and relatively low PDI values. MTS assay revealed that the nanoparticles do not induce alterations on viability of SH-SY5Y cells at lipidic concentrations bellow 0.25 mg/mL. These results highlight the potential of lipid-based nanoparticles to encapsulate MSCs' secretome and to function as brain-targeting delivery systems.

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Abstract **#640**

CYTOKINE SINKS: INJECTABLE HYDROGELS FUNCTIONALIZED WITH ANTIBODY FRAGMENTS NEUTRALIZING INFLAMMATORY AND PRO-CATABOLIC CYTOKINES

Lin Zhong[P][1], Michelle Koerselman[1], Lisanne Karbaat[1], Tom Kamperman[1], Jeroen Leijten[1], Bram Zoetebier[1], Marcel Karperien[1]

[1] *Department of Developmental BioEngineering, Faculty of Science and Technology, Technical Medical Centre, University of Twente*

Corresponding author's email: l.zhong-2@utwente.nl

Intra-articular (IA) injection with biologicals like cytokine neutralizing antibodies to treat osteoarthritis (OA) is challenging because of the rapid clearing of these biologicals from the joint space. To overcome these limitations, we developed a new concept that potentially can increase the therapeutic value of cytokine neutralizing antibodies in the management of OA. The concept is based on the use of injectable hydrogels functionalized with the variable domain of single chain heavy chain only antibodies (VHH) for cytokine capture. These hydrogels can either be pre-crosslinked in microgels before injection or injected as polymer-VHH conjugates that crosslink in a hydrogel in situ. To proof the concept of these so-called cytokine sinks we have selected a VHH that effectively neutralizes TNFα, which plays an important role in cartilage degradation in inflammatory arthritis. To achieve directed conjugation of the VHH to the polymer, recombinant DNA technology was used to introduce an unpaired cysteine in the C-terminus of the VHH. Hyaluronic acid was used as model polymer after substitution with tyramine and maleimide groups. We show successful conjugation of the VHH to the polymer backbone via thiol-maleimide chemistry. The VHH functionalized polymer could be used for making stable hydrogels after tyramine mediated crosslinking. Using an NF-κB luciferase reporter cell line we demonstrated that hydrogels functionalized with the VHH efficiently inactivated TNFα. Our results demonstrated that cytokine sinks have great potential for neutralizing inflammatory pro-catabolic cytokines after intra-articular injection by increasing the retention time of neutralizing antibody fragments in the joint cavity.

Keywords

Intra-articular; Hydrogels; VHH

Abstract **#642**

DEVELOPMENT OF SIRNA-ACTIVATED SCAFFOLD DELIVERY SYSTEM TO AMELIORATE INFLAMMATORY RESPONSES DURING REGENERATION OF OSTEOARTHRITIC CARTILAGE

Tom Hodgkinson[P][1], Domhnall Kelly[1], Claudio Intini[1], Brenton Cavanagh[1], James E. Dixon[2], Kevin M Shakesheff[2], Fergal J. O'Brien[1,3,4]

[1] *Royal College of Surgeons in Ireland, Dublin, Ireland*

[2] *University of Nottingham, UK*

[3] *Advanced Materials and Biomedical Engineering Research Centre (AMBER), Ireland*

[4] *Trinity Centre for Bioengineering, Trinity College Dublin, Ireland*

Corresponding author's email: tomhodgkinson@rcsi.com

In osteoarthritis, increased proinflammatory cytokine production in damaged joints hinders the success of biomaterial guided regenerative strategies. Recent evidence links the Hippo (YAP/TAZ) pathway with IL1B signalling[1]. Meanwhile data suggests that connexin membrane channel proteins have roles regulating gene/ protein expression and activity[2], including YAP[3] and NFkB[4]. Therefore, we investigated connexin-43 (Cx43) activity in chondrocyte responses to proinflammatory cytokines and the link between Cx43 and YAP-NFkB signalling. Then, we functionalized previously optimised chondrogenic collagen I-hyaluronic acid (CHyA) scaffolds [5] to develop a non-viral Cx43-siRNA biomaterial delivery platform targeting Cx43. In human articular chondrocytes (hACs), Cx43 and YAP-mediated responses to IL1B were investigated using small molecule inhibitors and promotors. The effects of Cx43 and YAP on cell viability, migration, proliferation and anabolic/ catabolic marker expression with IL1B stimulation were determined. For siRNA-activated scaffolds, Cx43 siRNA was complexed with the cell penetrating GET-(glycosaminoglycan-binding domain) peptide[6] for transfection, incorporated into CHyA scaffolds and the effect of Cx43 knockdown in hAC responses to IL1B assessed. IL1B decreased cell proliferation, migration and promoted catabolic marker expression. Cx43-channel inhibition and YAP promotion were found to attenuate this effect. Catabolic marker expression was significantly reduced in IL1B samples treated with Cx43 siRNA or YAP promotion. Scaffold delivery of GET-Cx43 successfully knocked-down Cx43 expression in hACs and decreased catabolic responses to IL1B. This study highlights links between Cx43, YAP and proinflammatory signalling related to osteoarthritis and demonstrates the potential for YAP1-Cx43 signalling as a therapeutic target. Scaffold-mediated GET-siRNA delivery demonstrates the potential for this approach in cartilage regeneration.

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Abstract **#643**

DEVELOPMENT OF A STARPEG-HEPARIN HYDROGEL SYSTEM TO PROMOTE CONTROLLED AND SUSTAINED DELIVERY OF ADIPOSE TISSUE DERIVED STEM CELLS' SECRETOME FOR CNS REGENERATIVE MEDICINE

Deolinda Silva$_P$[1,2,3], Passant Atallah[4], Lucas Schirmer[4], Ayham Frra[4], Tiffany Santos Pinho[1,2,3], António José Salgado[1,2], Carsten Werner[4,5]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

[2] *ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal*

[3] *Stemmatters, Biotecnologia e Medicina Regenerativa SA, Guimarães, Portugal*

[4] *Leibniz Institute of Polymer Research Dresden (IPF), Max Bergmann Center of Biomaterials Dresden (MBC), Dresden, Germany*

[5] *Technische Universität Dresden, Center for Regenerative Therapies Dresden (CRTD), Dresden, Germany*

Corresponding author's email: id7403@alunos.uminho.pt

INTRODUTION: Spinal Cord Injuries (SCI) affect both motor and sensorial functions of the body. One of promising approaches to tackle regeneration lies on the development of drug delivery systems that could increase bioavailability of therapeutic agents at lesion site, protecting it from degradation. Herein we propose the delivery of Adipose-derived stem cells (ASCs) secretome by in situ forming hydrogels made of star-shaped poly (ethylene glycol) (starPEG) crosslinked with heparin (Hep). Hep component provides affinity to cytokines, chemokines and growth factors and thereby prolonged release of secretome [1].

METHODS: ASCs secretome was loaded in a 1:1 ratio and the release profile evaluated by fluorescence intensity over 21 days as well as analytes detection by multiplex assay for 10 days. Moreover, mechanical properties of hydrogels were accessed such as rheology, mesh size and swelling ratio. Bioactivity effect of released secretome was evaluated by the capacity to promote differentiation of Neural Progenitor Cells (NPCs).

RESULTS: Fluorescence assays revealed that secretome was sustainably released for 21 days, being the highest amount released in the first 10 days. The multiplex assay allows the detection of Gal-1 released over 10 days. Regarding mechanical properties, hydrogels stiffness could range 500Pa-3000Pa, swelling ratio 1-2 and mesh size 10-20 nm. In vitro assays with NPCs show an increase of differentiated cells when loading secretome compared with vehicle.

CONCLUSIONS: Our reported data indicate that starPEG-heparin hydrogels are a suitable system to promote controlled and prolonged release of secretome. Hydrogel characteristics could be modulated to improve implantation, promoting SCI regeneration.

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Abstract **#644**

AUTOMATED GENERATION OF CUSTOMIZED ORGAN-ON-CHIP DEVICES

Pedro Costa[P][1]

[1] *BIOFABICS*

Corresponding author's email: pedro.costa@biofabics.com

Biofabrication is increasingly seen as the ideal solution for generating in vitro models. Not only it allows to accurately deposit and assemble specific materials (including cells) into complex 3D constructs but also allows to culture, stimulate, and analyze them into highly controlled and dynamic environments. Given the complexity of the human/animal body, it becomes necessary to develop tools which can automatically generate body-mimicking architectures and environments, particularly in a body-on-chip fashion. The purpose of this work was to demonstrate the limitless ability of newly developed software tools to generate body-like structures and environments and in this way democratize the access to organ-on-chip technologies.Parametric design methodologies were employed in order to enable automated generation of 3D device designs upon insertion of user-defined settings. Resulting parametrically designed shapes were then physically materialized by employing additive manufacturing and bioreactor know-how in order to produce fully functioning organ-on-chip devices fort testing under various conditions.It was possible to demonstrate that parametric design methodologies combined with additive manufacturing and bioreactor technologies may allow to easily, intuitively and automatically generate organ-on-chip devices with endless degrees of complexity, which may be employed in a wide array of applications.This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 828835.

Keywords

3D Software; 3D bioprinting; 3D models

Abstract **#646**

MSC SECRETOME DECREASES PRO-INFLAMMATORY RESPONSE IN LOADED AF ORGAN CULTURES

Graciosa Q Teixeira$_P$[1], Andreas Ekkerlein[1], Raquel M Goncalves[1,2], Joana R Ferreira[2], Yana Höpfner[1], Julia Nothelfer[1], Anita Ignatius[1], Hans-Joachim Wilke[1], Cornelia Neidlinger-Wilke[1]

[1] *Institute of Orthopaedic Research and Biomechanics, Ulm University, Ulm, Germany*

[2] *I3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal*

Corresponding author's email: graciosa.teixeira@uni-ulm.de

Introduction: Mesenchymal stem/stromal cells (MSC)-based therapies have been proposed for back pain and disc degeneration, despite limited knowledge on their action mechanism. Hence, we investigated the impact of a cell-free therapeutic approach with MSC secretome on AF organ cultures (AF-OCs) exposed to mechanical overload and proinflammatory environment [1].

Methods: AF-OCs were exposed to upper-physiological cyclic tensile strain and interleukin (IL)-1β (CTS+IL-1β) in a custom-made device [1]. A sub-group of stimulated AF-OCs was treated with human MSC secretome produced by preconditioned MSC (10 ng/mL IL-1β and 6% O2 for 48h). AF cells were characterized for gene expression changes. IL-6, MMP3 and collagen and AF adhesive strength were quantified in the tissue. Statistics: Kruskal-Wallis.

Results: A 4-days treatment of the CTS+IL-1β stimulated AF-OCs with MSC secretome downregulated the expression of inflammation markers (IL-6, IL-8), and metalloproteinases (MMP-1, MMP-3), but also of collagen type I ($p<0.05$). At protein level, it was confirmed that IL-6, MMP-3 and collagen content were decreased in the AF-OCs treated with the MSC secretome ($p<0.05$), as well as the annular adhesive strength ($p<0.05$).

Conclusion: The MSC secretome contributed to a decrease of the inflammatory and catabolic status of AF cells activated by CTS+IL-1β, and to the regulation of the complement system activation. However, it also contributed to a further decrease of collagen at gene/protein level and of the AF mechanical strength observed in the CTS+IL-1β group. The use of MSC secretome as therapeutic approach for disc-related diseases requires further mechanistic investigations before clinical trials.

Keywords

disc degeneration; mesenchymal stem cells secretome; inflammation

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Abstract **#647**

A COMPARISON OF MACROPHAGE PHENOTYPE AND CALCIFICATION INDUCED BY TWO BIOMATERIALS USED FOR CARDIOVASCULAR BIOENGINEERING

Madeline Cramer[P][1,2], Jordan Chang[1,2], Hongshuai Li[1], Aurelie Serrero[3], Mohammed El-Kurdi[3], Martijn Cox[3], Frederick Schoen[4,5], Stephen Badylak[1,2]

[1] *University of Pittsburgh*

[2] *McGowan Institute for Regenerative Medicine*

[3] *Xeltis BV*

[4] *Brigham and Women's Hospital*

[5] *Harvard Medical School*

Corresponding author's email: mcc116@pitt.edu

The host immune response to an implanted biomaterial, particularly the phenotype of macrophages, is a key determinant of the downstream remodeling outcome [1]. Endogenous tissue restoration (ETR) is a process by which an acellular device becomes infiltrated by host cells, is gradually resorbed and remodeled into autologous tissue, and remains functional throughout [2]. Successful application of ETR requires a host response that supports appropriate tissue remodeling. Therefore, design of materials to support ETR is an important area of investigation. An electrospun polymer scaffold with the supramolecular 2-ureido-4[1H]-pyrimidinone (UPy) binding motif (ePC-UPy) based on the RestoreXTM material platform has been developed by Xeltis BV (Eindhoven, The Netherlands) for ETR-based repair of cardiovascular tissue [3–5].The present study compared the macrophage phenotype response, remodeling and calcification properties of the ETR-based ePC-UPy scaffold and a glutaraldehyde-fixed bovine pericardium (GF-BP) biologic scaffold, used widely in heart valve replacement.

The ePC-UPy material induced infiltration of mononuclear cells throughout the thickness of the scaffold within 2 days and neovascularization at 14 days. GF-BP elicited a balance of pro-inflammatory (M1-like) and anti-inflammatory (M2-like) macrophages, while ePC-UPy supported a dominant M2-like macrophage phenotype at all timepoints. Relative to GF-BP, ePC-UPy was less susceptible to calcification for the 180 day duration of the study. The results of this study highlight the divergent macrophage and remodeling response to distinct biomaterials. The favorable remodeling outcome induced by ePC-UPy (as indicated by a high M2-like:M1-like ratio) suggest that this material platform may be suitable for ETR-based heart valve replacement and other clinical applications.

Keywords

macrophage; endogenous tissue restoration; supramolecular polymer

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Abstract **#648**

DEVELOPMENT OF A 3D HUMAN INDUCED PLURIPOTENT STEM CELL SPINAL CORD SCAFFOLD SYSTEM TO INVESTIGATE AND PROMOTE SPINAL CORD REPAIR

Cian O' Connor[1,2], Ian Woods[1,2], Sean Kerr[1], Adrian Dervan[1,2], Fergal O'Brien[1,2]

[1] *Tissue Engineering Research Group, Dept. of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland*

[2] *Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland*

Corresponding author's email: cianoconnor@rcsi.com

Following spinal cord injury, trophic cells called astrocytes, become 'reactive'[1] and contribute to scar formation[2], preventing injured neurons from growing axons through the lesion site to restore sensorimotor function. Currently, no full therapeutic exists to facilitate cord repair due to the poor understanding of the underlying cellular mechanisms post-injury, and the lack of supportive environment to promote recovery[2]. Building on expertise in developing peripheral nerve guidance scaffolds[3] we aimed to create a biomimetic scaffold for 3D cord modelling and to provide a trophic environment for repair. Screening of native central nervous system (CNS) extracellular matrix (ECM) proteins revealed that collagen-IV and fibronectin combined, enhanced motorneuron (39%, $p<0.01$) and spinal cord astrocyte outgrowth (70%, $p<0.05$). Subsequent seeding of hyaluronic acid scaffolds functionalized with both ECM proteins, with mechanical properties and aligned microarchitecture similar to the spinal cord, with induced pluripotent stem cell (iPSC) derived astrocytes and neurons showed that soft scaffolds (1.9kPa) promoted viability ($p<0.05$), outgrowth/infiltration ($p<0.0001$) and differentiation compared to stiffer scaffolds (3kPa & 6.1kPa). Furthermore, soft scaffolds encouraged growth of iPSC derived sphereoids that subsequently formed extensive neuronal/astrocytic networks with distinct Beta-tubulin III+ (neurons) and GFAP+ (astrocytes) processes that connected with other spheroids. Here we show the creation of a novel scaffold environment that supports robust iPSC derived astrocytic and neuronal growth. These findings have implications for 3D modelling of astrocyte-neuronal interactions following spinal cord injury and further development of scaffold therapeutics for promoting spinal cord repair. Research funded by IRFU charitable trust, Anatomical Society UK and SFI-AMBER centre.

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Abstract **#649**

A ZEOLITE-MODIFIED PEEK POLYMER PROMOTES A M2-LIKE MACROPHAGE PHENOTYPE AND ENHANCES THE OSTEOBLASTIC DIFFERENTIATION OF PROGENITOR CELLS

Arthi Shridhar[P][1,2], Kelsey Hall[1,2], Joseph Bartolacci[1,2], Stephen Badylak[1,2]

[1] *University of Pittsburgh, School of Medicine*

[2] *McGowan Institute of Regenerative Medicine*

Corresponding author's email: ars307@pitt.edu

Poly-ether-ether-ketone (PEEK) and titanium, commonly used in orthopaedics often elicit a pro-inflammatory phenotype among cells of the innate immune system1. With the immune system playing a crucial role in modulating inflammation and bone homeostasis, alternatives that attenuate the pro-inflammatory environment and promote osseointegration are required2. The present study compares the in vitro response of (i) murine macrophages and (ii) SAOS-2 cells when cultured on a PEEK-zeolite composite material (ZFUZE) versus PEEK and Titanium substrates (DiFusion Inc, Austin, TX). Primary bone marrow-derived derived macrophages (2x106 cells) were seeded on each substrate and differentiated for 7 d. Macrophage phenotype was evaluated using RT-qPCR for pro-inflammatory (iNOS, IL1β and IL6) and anti-inflammatory (KLF4, Arg1 and Fizz1) markers, and confirmed with immunolabelling. To assess osteogenesis, SAOS-2 cells were seeded at a density of 1x104 cells/cm2. At confluence, gene expression was evaluated by RT-qPCR (BMP2, BMP4 and OCN), protein expression was assayed in the conditioned medium using ELISA (BNP2, BMP4, BMP7 and OCN) and alkaline phosphatase (ALP) enzyme activity was determined in cell lysates. The ZFUZE and PEEK materials reduced the gene expression of pro-inflammatory markers (IL1B & IL6) and anti-inflammatory marker, KLF4 compared to Titanium. Immunolabeling, indicated a pronounced (Fizz1+) M2-like phenotype in macrophages cultured around ZFUZE samples. Preliminary osteogenic data showed augmented BMP4 gene expression and BMP2 protein expression on ZFUZETM articles compared to all other groups. Taken together, the present study suggests that ZFUZETM represents a promising alternative orthopedic substrate that promotes a favourable host immune response, and could potentially enhance osseointegration.

Keywords

Macrophage; Osteoimmunology; Osteogenesis

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Abstract **#650**

3D-PRINTED COMPOSITE TUBULAR SCAFFOLDS FOR TRACHEAL TISSUE REGENERATION WITH HIGH POTENTIAL FOR SUBMUCOSAL VASCULARISATION

Tehreem Khalid[P][1,2,3], Mark Lemoine[2,3,4], Sally-Ann Cryan[1,2,3], Fergal O'Brien[2,3,4], Cian O'Leary[1,2,3]

[1] *School of Pharmacy and Biomolecular Sciences, RCSI, Dublin, Dublin, Ireland.*

[2] *Tissue Engineering Research Group, RCSI, Dublin, Ireland.*

[3] *Advanced Materials & Bioengineering Research (AMBER) Centre, RCSI, Dublin, Ireland.*

[4] *Trinity Centre for Biomedical Engineering, TCD, Dublin, Ireland.*

Corresponding author's email: tehreemkhalid@rcsi.com

INTRODUCTION

Attempted tracheal replacement efforts to date have had very little success, often succumbing to stenosis within poorly-vascularised constructs[1]. The major objective of this study was to redevelop a 3D in vitro collagen-hyaluronate scaffold[2] as an innovative tracheal medical device by increasing its mechanical properties and maximising submucosal blood vessel formation.

METHODS

A biodegradable, thermoplastic polymer was used to 3D-print (3DP) a tubular backbone for integration into lyophilised collagen-hyaluronate biomaterials, with scaffold design optimised via multimodal mechanical assessment. The construct's vascularisation potential was determined by using a previously established in vitro co-culture model[3] of HUVECs and human MSCs. Biocompatibility, vessel formation, and angiogenic marker expression were all analysed.

RESULTS

The 3DP backbone was successfully incorporated by lyophilisation into collagen-hyaluronate scaffolds, producing a composite with pores of 300µm for ideal vascularisation[3]. The backbone provided sufficient scaffold strength, with compressive moduli of 0.09-0.19 MPa, and flexural moduli of 0.13-0.29 MPa, all mimicking native trachea[4]. Cellular metabolic activity and live-dead staining indicated that the 3DP synthetic backbone did not have a detrimental effect on cellular viability. Furthermore, the 3DP scaffolds supported the expression of angiogenic markers vascular endothelial growth factor and basic fibroblast growth factor, as well as the formation of vessel-like structures in 3DP scaffolds.

DISCUSSION & CONCLUSION

By addressing both the mechanical and vascularisation requirements of a tracheal scaffold, this work has begun to pave the way for a new therapeutic option to resolve the shortcomings of current treatments.

ACKNOWLEDGMENTS

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Keywords

Respiratory ; 3D-printing; Medical Device

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Abstract **#651**

AUTOMATION OF FIBRE PATTERNING IN 3D FOR CELL-CULTURE DEVICES PRODUCTION

Ruishan Liu[P][1], Elisabeth Lauren Gill[1], Yan Yan Shery Huang[1]
[1] *Department of Engineering, University of Cambridge, UK*
Corresponding author's email: rl614@cam.ac.uk

The electrospun fibrous scaffolds have gained popularity recently. Limited methods exist to apply low voltage and actual automation of patterning fibres in three dimensions. Hence, it is envisioned that automaton of patterning fibres in three dimensions with low electrospinning voltage would be realised, and thus mass production of fibrous scaffolds would be achieved. We have fabricated three-dimensional fibrous devices batch by batch via additive manufacturing (particularly fused filament fabrication technique) and electrospinning (particularly near field low-voltage electrospinning patterning technique). Consequently, polylactic-acid initiator and gelatine fibres (with 3-5 um diameter and 150 um inter-fibre pitch) could be integrated automatically without manual stacking. This integrated scheme would reduce capital and operating costs, and safety hazards while scaling up production. We have seeded cells (fibroblast cell line and brain cancer cell line) onto the three-dimensional fibres. Results have demonstrated the biocompatibility of the cell-culture devices since cell attachment and proliferation have been observed. We have applied machine learning (regression models) to find the correlations among operating parameters and product quality. Machine learning based on regression models could offer advantages, including accurate prediction and parameter optimization. Future work involving multi-functional fibres deposition is proposed to accelerate the application of fibrous devices in tissue engineering and drug discovery areas.

Abstract **#652**

REMOTE CONTROL HEALING FOR OA REPAIR

Iria Echevarria$_P$[1,2], Hareklea Markides[1], Alicia El Haj[1]

[1] *University of Birmingham*

[2] *Keele University*

Corresponding author's email: I.Echevarria@bham.ac.uk

The clinical need for improving the limitations of the current tissue engineering approaches for OA repair could be addressed by the use of novel technologies and allogenic cell sources. Mechanical regulators are known to play a key role in osteoarthritis and present some key targets for modulation. The mechanotransductive potential of stem cells introduces the possibility of using external mechanical stimuli to promote cell differentiation towards a chondrogenic pathway and provide cues for tissue maturation. Our group has focused on the use of MNPs to remotely control the stem cell fate by modulation of the mechanical regulators of the cells1.

MNPs were functionalized with TRPV4 antibodies, to target the mechanosensitive ion-channel of human Umbilical Cord-derived stem cells (UC-SCs) from 3 donors. Labelled cells were cultured as pellets for 21 days in chondrogenic media and submitted to 1h daily magnetic stimulation regimes using the MICA magnetic bioreactor. Chondrogenesis was evaluated by histological staining showing enhanced deposition for all the chondrogenic markers on MICA activated groups, and immunohistochemistry that showed a sustained proliferation of MICA groups together with SOX9 and COL2 early expression and a low signal of COLX. These results were supported by gene expression analysis and biochemical tests.

MICA activation enhances chondrogenic response on UC-SCs by an increased matrix deposition and enhanced cell growth and proliferation. The mechanical activation on MSCs also induces an early response on COL2 production. TRPV4 is showed to be a specific mechano-inducible target that triggers a chondrogenic response when activated remotely with MNPs.

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Abstract **#653**

MATRIX-BOUND NANOVESICLES PRESERVE RETINAL GANGLION CELL INTEGRITY AND FUNCTION FOLLOWING ISCHEMIC INJURY

Jordan Chang[P1,2], Yolandi van der Merwe[2,3], Anne Faust[2,3], Ecem Sakalli[2], Caroline Westrick[2], George Hussey[2], Kevin Chan[3], Ian Conner[3,2], Valeria Fu[3], Stephen Badylak[2], Michael Steketee[3,2]

[1] *University of Pittsburgh School of Medicine*

[2] *McGowan Institute for Regenerative Medicine*

[3] *Department of Ophthalmology, University of Pittsburgh*

Corresponding author's email: jtc60@pitt.edu

Injury to retinal ganglion cells (RGC) as a result of acute trauma or progressive insult such as occurs with glaucoma can lead to axon degeneration and irreversible loss of visual function [1]. The present study shows that Matrix Bound Nanovesicles (MBV), a distinct class of extracellular vesicles localized to extracellular collagen fibrils [2], mitigate the inflammatory response and preserve RGC viability and function following experimentally induced severe acute intraocular pressure (IOP) elevation in a rat model. Intravitreal MBV injection attenuated IOP-induced RGC axon degeneration and cell death, protected RGC axon connectivity to visual nuclei in the brain, and prevented loss of retinal functioning as shown by histology, anterograde axon labeling, manganese-enhanced magnetic resonance imaging, and electroretinography. In the optic nerve, MBV also prevented IOP-induced decreases in growth-associated protein-43, a neuron-specific marker up-regulated in regenerating axons [3], and elevations in levels of glial fibrillary acidic protein, suggesting decreased astrocyte activation consistent with RGC health [4]. In vitro studies showed MBV mitigated the pro-inflammatory phenotype of activated microglia and astrocytes, stimulated RGC neurite growth, and protected RGC from conditioned media generated by activated astrocytes while exhibiting no cytotoxic effects on RGC viability. Thus, MBV can modulate distinct signaling pathways (e.g., inflammation, cell death, and axon growth) in diverse cell types. Since MBV are naturally-derived structures and contain bioactive factors present in numerous FDA approved devices, MBV may be readily useful, not only experimentally, but also clinically as an immunomodulatory, neuroprotective approach for treatment of ocular trauma or disease and other CNS tissues.

Keywords

Matrix-Bound Nanovesicles; Immunomodulation; Ischemia

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Abstract **#656**

FUNCTIONALISED MAGNETIC NANOPARTICLES FOR PROMOTION OF WNT SIGNALLING ACTIVATION AND NEURITE OUTGROWTH AND MIGRATION

Kiran Dhillon[P][1], Michael Rotherham[2], Afeesh Rajan Unnithan[1], Neil Telling[2], Alicia El Haj[1]

[1] *University of Birmingham*

[2] *Keele University*

Corresponding author's email: k.dhillon.1@bham.ac.uk

Parkinson's disease (PD) is characterised by the loss of midbrain dopaminergic neurons within the substantia nigra, responsible for dopamine regulation via their projections through the nigrostriatal pathway that connects to the striatum(1). Thus, reconstruction of this crucial pathway is essential for improving cell transplantation therapies for PD. Wnt signalling is an important developmental pathway that regulates dopaminergic cell proliferation and differentiation(2). Here we propose methods to promote and study both neurite outgrowth, migration and Wnt activation(3) using magnetic nanoparticles in 2D and 3D experimental models to assess magnetic field stimulation as a remote activator.

Magnetic nanoparticles (MNPs) were functionalised and coated with a peptide, UM206 (which binds to Wnt Frizzled receptors), and used to label the dopaminergic neuronal cell line, SH-SY5Y. An oscillating magnetic field was applied and expression of dopaminergic markers were assessed via immunocytochemistry. MNPs were also coated with graphene-oxide and internalised in cells PC-12 and SH-SY5Y cells, using P21-8R(4), a transfection agent. 3D collagen-based hydrogel models were created and optimised in which neuronal differentiation of graphene-oxide MNP labelled cells was induced during the application of a static magnetic field. Neurite outgrowth and migration was investigated using confocal imaging and immunocytochemistry.

Results indicate that UM206 coated MNPs showed successful activation of Wnt signalling and subsequent increase of dopamine expression markers. The 3D hydrogel systems showed promising potential as useful and relevant models to investigate neuronal migration and outgrowth. The use of MNPs and their functionalisation can be considered encouraging tools in remote control of cellular functions and behaviour.

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Abstract **#657**

MULTISCALE VASCULARIZED CARDIAC PATCHES VIA COMBINED 3D BIOPRINTING AND MICROPHYSIOLOGICAL CONDITIONING

Michael Daniele[P][1,2], Parthiban Rajan[1], Frances S. Ligler[1,2], Ke Cheng[1,2]

[1] *NC State University*

[2] *University of North Carolina at Chapel Hill*

Corresponding author's email: mdaniel6@ncsu.edu

Engineering multiscale vascular networks is a key challenge in biofabrication. The hierarchical, microscale anatomy plays a crucial role in enabling connectivity to external perfusion sources and ensuring transplant's grafting success. Decoupling the multiscale problem of engineering vascular networks, i.e. directing the mesoscale vessel formation while providing for de novo formation of microscale vessels, is a viable strategy to reduce the necessary spatial resolution, processing time, and complexity of the biofabrication process. We focus on establishing a protocol for repeatable microfabrication of 3D cell-laden hydrogel scaffolds with arteriole and venule scale microvasculature, which can be further expanded into a capillary bed by conditioning with pulsatile perfusion on a microfluidic platform. We demonstrate the simple 3D bioprinting of vascularized cardiac patches with a custom digital light processing (DLP) bioprinter. The vascularized cardiac patches are composed of human coronary artery endothelial cells and cardiosphere-derived cardiac stromal cells. The vascularized cardiac patches are then conditioned in microfluidic device, which regulates pulsatile perfusion through the bioprinted vascular network, delivers growth factors, and monitors vascularity by the measurement of the hydrodynamic resistance of the tissue construct. These conditioned, vascularized cardiac patches are evaluated for efficacy in treating myocardial infarction. Integration with the myocardium, promotion of regeneration, and subsequent cardiac function are characterized.

Abstract **#658**

ESTABLISHING A PROTEIN-BASED MULTIPLEX SCREENING TOOL FOR CALCIUM PHOSPHATE–BASED BIOMATERIALS

Maria José Eischen-Loges[P][1], Zeinab Tahmasebi Birgani[1], Mireille Sthijns[1], Yousra Alaoui Selsouli[1], Hoon Rho[1], Vanessa LaPointe[1], Pamela Habibović[1]

[1] *Maastricht University*

Corresponding author's email: maria.eischen-loges@maastrichtuniversity.nl

Introduction: Calcium phosphate (CaP)–based biomaterials are widely used in bone regeneration applications [1]. Nevertheless, their properties and clinical performance remain inferior to their biological counterpart, i.e. autologous bone graft. Hence, there is a need for further improvement [2]. We propose that a lack of reliable in vitro screening methods for such biomaterials has hampered our ability to find an optimal material. Here, we present a multiplex protein assay as a high-throughput tool for screening the osteogenic properties of CaPs and for predicting their success in promoting osteogenic differentiation.

Methods: In order to establish the osteogenic differentiation programme of human mesenchymal stromal cells (hMSCs), they were cultured in basic medium and osteogenic medium with or without the addition of BMP-2, in basic medium supplemented with Ca2+ or PO43-, and on beta-tricalcium phosphate (TCP) discs. A multiplex protein assay to detect analytes related to bone formation, resorption, angiogenesis, and immunomodulation was performed at multiple time points. Osteogenic differentiation was confirmed by conventional methods such as Alizarin red stain, alkaline phosphatase assay, and qPCR.

Results and discussion: The protein profile for hMSCs cultured in the different medium conditions, with the addition of Ca2+ or PO43-, and on TCP discs varied over time. With a statistical model, we correlated the protein expression to the results of osteogenic differentiation for a descriptive panel of markers.

Conclusion: Here, we presented our efforts towards developing a high-throughput screening tool based on protein multiplex for evaluating osteogenic properties of CaP-based biomaterials.

Keywords

Protein Multiplex; Human mesenchymal stem cells; Calcium phosphate

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The **Fraunhofer IPT** in Aachen has extensive experience in the development of automated production facilities for the manufacture of stem cell-based products and gene and cell therapeutics. Current project examples are: AutoCRAT, AIDPATH, JointPromise, BellaFactum and AIxCell. Since 2010, competencies in the field of automation of biopharmaceutical processes have been gathered, such as the development of concepts and systems for the automated production of therapeutics and the integration of measurement technology and sensors for adaptive process control. In addition, own solutions have been developed for "technology gaps", such as high-speed microscopy for automated quality control of cell culture processes. There is also deep knowledge in the field of process control (projects: AIMFREE, FreeMove, FabOS) as well as the connection of heterogeneous machine landscapes to a production platform in the GMP environment, the creation of digital concepts for model predictive monitoring and control of the process.

Your Contact:
M.Sc. Bastian Nießing
Group Manager
Automation in Life Sciences
+49 241 8904 142
Bastian.niessing@ipt.fraunhofer.de

Fraunhofer
IPT

Abstract **#659**

HMSCS RESPONSE TO POLYMER-CALCIUM PHOSPHATE COMPOSITE SCAFFOLDS CONTAINING ZINC PRODUCED USING ADDITIVE MANUFACTURING

Martyna Nikody[P][1,2], Jiaping Li[1,2], David Koper[1,2,3], Elizabeth Rosado Balmayor[1], Lorenzo Moroni[2], Pamela Habibovic[1]

[1] *Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands*

[2] *Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands*

[3] *Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Center, Maastricht, the Netherlands*

Corresponding author's email: m.nikody@maastrichtuniversity.nl

Currently available options for treating critical-size craniomaxillofacial bone defects involve the use of either autologous bone or, alternatively, of a bioinert bone graft substitute. However, the use of autologous bone can result in complications including donor site morbidity, while bioinert materials lack osteointegration and have a high risk of implant failure (1). We propose the use of a novel bioactive composite with high ceramic content composed of poly(ethyleneoxide terephthalate)/poly(butylene terephthalate) (1000PEOT70PBT30, PolyActive, PA) and 50% beta-tricalcium phosphate (β-TCP) with the addition of zinc (Zn) in a form of a coating of the TCP particles. Several inorganic ions have been shown to affect biochemical functions important for different aspects of bone regeneration (2). Although Zn is found in the bone only in trace amounts, it plays an essential role in its metabolism by stimulating bone formation by osteoblasts and inhibiting osteoclasts differentiation (3). Therefore, we hypothesise that the addition of zinc to the β-TCP will result in enhanced osteogenic properties.

Overall, human mesenchymal stromal cells (hMSCs) cultured on all types of scaffolds showed cell growth and alkaline phosphatase (ALP) production. Higher cell growth and ALP production were observed on scaffolds containing β-TCP and β-TCP with the addition of zinc than on 1000PEOT70PBT30 scaffolds. Furthermore, extracellular matrix formation was observed on all scaffolds except for 1000PEOT70PBT30 scaffolds. The osteogenic properties of tested scaffolds will be further investigated by evaluation of cell differentiation at mRNA level by qPCR analysis for a set of specific osteogenic markers.

Keywords

Bone regeneration; Calcium phosphate; Zinc

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Abstract **#660**

HYBRID BIOINKS CONTAINING LIQUEFIED BIOFACTORIES

Maryam Ghasemzadeh-Hasankolaei[1], Clara R. Correia[1], João F. Mano[1]
[1] *CICECO–Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*
Corresponding author's email: maryam.gh@ua.pt

The aim of this work is the development of a natural-based bioink enriched with liquefied living microfactories. These living microfactories consist of liquefied compartments encapsulating stromal cells and surface-modified microparticles. Such a system has been proved as a suitable cell encapsulation strategy for tissue engineering [1]. Herein, the novelty consists in the combination of the liquefied encapsulation technology with an external and supportive hydrogel to confer bioprintability to the system and produce clinically relevant tissue engineered constructs. Using the electrospraying technique, alginate-microbeads containing adipose-derived stromal cells and surface-modified microparticles were produced. Then, alginate-microbeads were combined with gelatin methacryloyl or methacrylated hyaluronic acid or a combination of thereof, to produce hybrid bioinks. After bioprinting, results showed that we successfully developed stable hybrid bioinks even after the liquefaction process. Biological assays in all tested bioinks indicated that the viscoelastic structure of solid alginate-microbeads during the bioprinting process, as well as the liquid microenvironment provided after liquefaction, allowed cells to remain viable. More importantly, surface-modified microparticles offered proper support for cell interaction and structural organization. Overall, this study developed a proof-of-concept strategy which can be applied for bioprinting of any kind of anchorage-dependent cells. The versatility of this technique facilitates engineering of desired niches for encapsulated cells, while the developed bioink can be designed with optimal mechanical properties without cellular bio-performance constrains.

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Keywords
surface-modified microparticles; hybrid bioink; anchorage-dependent cells

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Abstract **#661**

SELF-SUPPORTING LAMINARAN-BASED HYDROGELS

Mehrzad Zargarzadeh[P][1], Cláudia Nunes[1], Manuel A. Coimbra[1,2], Catarina A. Custódio[1], João F. Mano[1]

[1] Department of Chemistry, CICECO - Aveiro Institute of Materials, University of Aveiro, 3810-193, Aveiro, Portugal

[2] LAQV-REQUIMTE, Department of Chemistry, University of Aveiro

Corresponding author's email: mehrzad.zargarzadeh@ua.pt

Hydrogels as Extracellular Matrix (ECM)-mimic systems have revealed exceptional promises to support the 3D encapsulation of cells towards the development of tissue-engineered constructs. However, in some cases, the use of bulk hydrogels is associated with the limitation in providing an adequate supply of oxygen and nutrients, in particularly glucose as the main source of energy and metabolic intermediate in living cells [1, 2].

Recently laminaran(laminarin), a β-glucan storage polysaccharide, have been applied for the development of photo-cross-linkable hydrogels [3]. Here we report a platform for cell culture that takes advantage of the degradation process of the laminaran hydrogels. The degradation mechanism of choice is enzymatic as it allows the release of glucose by gradual degradation of the polymer by specific hydrolyzing enzymes.

We fabricate the enzyme encapsulated laminaran hydrogels to obtain structural platforms for 3D cell culture but also achieving a satisfying release of glucose over a prolonged period in order to enhance the cells survival. We demonstrated that 3D encapsulated tumor cells (A549) and human mesenchymal stem cells (hMSCs), exhibited improved survival rate and proper functionality due to the beneficial properties of the glucose production.

We believe, this self-supporting bioscaffolds with enzyme-empowered degradation capacity can successfully overcome glucose diffusion limitation. Such innovation is expected to circumvent the limitations of the current hydrogels strategies that lack on nutrients diffusion and boosting the application of hydrogels in a variety of bioengineering applications including in tissue engineering.

Keywords

Self-feeding hydrogels; Enzymatic hydrolysis; Laminaran

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Abstract **#663**

AN INTEGRATIVE APPROACH IN DEVELOPING SCAFFOLDS BASED ON GELLAN GUM AND BIOACTIVE GLASS AIMED FOR OSTEOCHONDRAL TISSUE ENGINEERING

Jovana Zvicer[P1], Mia Radonjic[1], Ilijana Kovrlija[1], Ana Medic[2,3], Sasa Novak[2,3], Bojana Obradovic[1]

[1] *Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

[2] *Department for Nanostructured Materials, Jožef Stefan Institute, Ljubljana, Slovenia*

[3] *Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*

Corresponding author's email: jzvicer@tmf.bg.ac.rs

Bilayer scaffolds based on gellan gum (GG) and nanoparticulate bioactive-glass (BAG) were developed by an integrative approach based on engineering principles and characterization in biomimetic bioreactors. The osteo-inductive GG-BAG layer containing 2 % w/w GG and 2 % w/w BAG (composition: 70 n/n % SiO2, 30 n/n % CaO) was produced by gelation followed by freeze-drying to obtain open porosity in axial and radial directions. The chondral layer was obtained by dispensing a warm 2 % w/w GG solution at 60˚C over the frozen macroporous GG-BAG layer at -25˚C. The temperatures were optimized by applying a one-dimensional unsteady-state heat transfer model so to obtain a thin integration zone, 0.5 – 1 mm thick. The scaffolds were evaluated regarding bioactivity in a biomimetic bioreactor with specially designed chambers to provide supply of two media relevant for chondral and bone tissues. In the present experiment, simulated body fluid (SBF) was supplied countercurrently continuously during 14 days of the experiment (1.1 ml min-1 flowrate), while dynamic compression (5 % deformation, 0.68 Hz frequency, 337.5 µm s-1 loading rate, 1 h / day) was applied on the chondral layer, from day 7 to day 14. SEM analyses have confirmed the retained integrity of the scaffolds, as well as formation of hydroxyapatite (HAp) uniformly throughout the osteo-layer of the scaffolds.Significantly higher bioactivity under biomimetic conditions compared to static controls resulted in slightly but significantly increased compression modulus. These results indicated a high potential of the applied integrative strategy for the development of biomimetic bilayer scaffolds.

Abstract **#664**

CALCIFICATION OF TISSUE ENGINEERED PULMONARY VALVES IMPLANTED IN LARGE ANIMAL MODELS, A SYSTEMATIC REVIEW AND META-ANALYSIS

Dewy van der Valk[P][1], Marcelle Uiterwijk[2], Aleksandra Fomina[1], Anat Akiva[3], Anthal Smits[1], Carlijn Bouten[1]

[1] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands*

[2] *Heart Center, Amsterdam University Medical Center, Amsterdam, The Netherlands*

[3] *Department of Cell Biology, RadboudUMC, Nijmegen, The Netherlands*

Corresponding author's email: d.c.v.d.valk@tue.nl

Tissue engineered heart valves (TEHVs) are promising alternatives to non-living bio-prosthetic and mechanical valve prostheses, prospecting life-long replacement options that can adapt to a changing hemodynamic environment [1,2]. For truly safe translation to the clinic, some drawbacks need to be overcome [3], a major one being (micro)calcification of TEHVs.

In this systematic review, we analyzed calcification found in pulmonary TEHVs, implanted in large animal models. We studied the influence of tissue engineering methods on calcification and tissue-specific differences in histological differentiation of TEHVs. A total of 78 studies were included in data extraction, of which 38 studies containing 78 experimental groups could be included in meta-analysis. The overall calcification event rate (ER) was 0,35 (95%CI [0,28-0,43]), with a large heterogeneity between study outcomes. Calcification was more prone in the conduits (ER 0,34 [0,26-0,43]) than in the leaflets (ER 0,20 [0,16-0,26]). Some trends were observed, yet there were no significant differences in calcification between natural or synthetic scaffolds, cell-seeding methods, or different animal models and follow-up times.

This study showed that calcification is frequently found in large animal models for TEHVs. Control-based research could shed further light upon causes and solutions for calcification, bringing the field of TEHVs forward towards clinical use.

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Abstract **#665**

EVALUATION OF BIOMIMETIC COMPOSITE SCAFFOLDS BASED ON ALGINATE CONTAINING BIOACTIVE HYDROXYAPATITE PRECURSORS UNDER CONDITIONS RELEVANT FOR BONE TISSUE ENGINEERING

Jasmina Stojkovska[P][1,2], Jovana Zvicer[1], Milica Andrejevic[1], Djordje Janackovic[1], Bojana Obradovic[1], Djordje Veljovic[1]

[1] *Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

[2] *Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia*

Corresponding author's email: jstojkovska@tmf.bg.ac.rs

The aim of this study was to investigate possibilities for production of macroporous scaffolds based on alginate hydrogels with addition of hydroxyapatite (HAP) precursors and to evaluate the best candidates under biomimetic conditions in perfusion bioreactors for potential use in bone tissue engineering. HAP powders, undoped and doped with Mg2+, were synthesized using a hydrothermal method followed by calcination. Composite scaffolds with different fillers were prepared by an improved procedure including gelation and freeze-drying method. Scanning electron microscopy has shown uniformly dispersed mineral phase in all obtained highly macroporous composite scaffolds with open and connected pores. Biomechanical properties of all scaffolds were investigated in a biomimetic bioreactor with dynamic compression (0.28 Hz frequency, 10% deformation). Compressive strength of scaffolds increased with the increase in Ca2+ and the decrease in Mg2+ concentrations in fillers. The formation of HAP crystals within all scaffolds was examined in the simulated body fluid (SBF) during 28 days under static conditions, while the best candidate (Mg substituted HAP filler, precursor solution with (Ca+Mg)/P molar ratio of 1.52) was evaluated under physiologically relevant conditions in perfusion biomimetic bioreactors with the direct SBF flow at the superficial velocity of 400 µm/s. The continuous perfusion induced enhanced formation of HAP crystals throughout the scaffolds leading to improved mechanical properties as compared to the initial scaffolds. The results of this study have shown potentials of the macroporous scaffolds with incorporated nanostructured Mg substituted HAP fillers in conjunction with perfusion bioreactors as a biomimetic environment for application in bone tissue engineering

Abstract **#666**

FUNCTIONAL REGENERATION IN SPINAL CORD INJURY INDUCED BY THE SECRETOME OF ADIPOSE TISSUE DERIVED STEM CELLS

Jorge Ribeiro$_P$[1,2], Rita Silva[1,2], Susana Monteiro[1,2], Rui Lima[1,2], Sofia Serra[1,2], Fábio Teixeira[1,2], José Graça[1,2], Nuno Silva[1,2], António Salgado[1,2]

[1] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portuga*

[2] *ICVS/3B´s – PT Government Associate Laboratory, Braga/Guimaraes, Portugal*

Corresponding author's email: jorgecibraoribeiro@gmail.com

Spinal cord Injury (SCI) is a dramatic pathology with a high number of new cases emerging every year. The injury triggers several pathophysiological events which cause damage and supress axonal growth in the spinal cord tissue, causing motor, sensory loss and causing incapacitating conditions to the patients. In this work we have assessed the therapeutic value of adipose tissue derived stem cells (ASCs) secretome on two distinct models of SCI in rodents (transection and compression). In both models, the secretome was intravenously administered repeated times in acute and chronic phase with the animals showing improvements at motor, sensory and histological level . The locomotor performance evaluated in the BMS test shown a threefold increase in the groups treated with secretome over control in both injury settings. Moreover, the transection group treated with secretome significatively improved the sensory score evaluated in the Von Frey test compared with untreated animals. Reduction of inflammatory cells (microglia and macrophages) was also observed in the secretome treatment on both models compared with control, being the difference statically significant in the transection group. In this model the secretome also increased the expression of Gap-43 and beta-III tubulin markers when compared with control. This result translates in improved regeneration and axonal growth in the lesion site besides a decrease in the lesion cavities. Altogether, this provide evidences of the therapeutic potential of ASC secretome after SCI, supported by indications on the positive effects exerted on neuroinflammation, axonal outgrowth and regeneration, observed in two distinct models of SCI.

Abstract **#667**

THE SURFACE MODIFICATION OF 316L STAINLESS STEEL AND IN VITRO TESTS FOR CARDIOVASCULAR STENT APPLICATION

Yaşar Kemal ERDOĞAN[P][1], Batur ERCAN[1,2,3]

[1] *Department of Biomedical Engineering, Middle East Technical University*

[2] *Department of Metallurgical and Materials Engineering, Middle East Technical University*

[3] *BIOMATEN, METU Center of Excellence in Biomaterials and Tissue Engineering, Ankara, Turkey*

Corresponding author's email: yasarkemalerdogan@gmail.com

316L stainless steel was the material of choice for cardiovascular stent applications due to its mechanical properties, corrosion resistance and established manufacturing techniques. However, the use of bare stainless steel in clinics has been decreasing steadily. The leading factors for the decreased use of stainless steel include nickel ion release, limited endothelization, thrombus formation and restenosis. In this study, 316L SS surface were modified with anodization technique to obtained oxidized based nanopit structures with a size range from 25 to 250 nm in diameter. In addition, we altered the surface morphology of the nanopit features to have higher depths and thicker oxide layer on 316L stainless steel via two-step anodization. Surface topography of the samples showed that root mean square roughness (rms) value for non-anodized sample was 1.2 nm, while 200 nm surfaces had an rms value of 17.2 nm. XPS results showed that anodized surfaces were composed of Fe2O3, Cr2O3 and O1s. We observed Ni peak intensity to decrease as diameter of nanostructures increased. Also, nickel ion release from anodized and non-anodized surfaces at 37°C for 30 days were assessed with ICP-MS. The in vitro tests revealed that nanostructured surfaces promoted adhesion and proliferation of human umbilical vein endothelial cells (HUVECs). Especially, 200 nm of nanopit surface was promising for rapid re-endothelialization and could possibly minimize problems of bare metal for stent applications.

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Abstract **#669**

CHARACTERISATION OF A POLYCAPROLACTONE ELECTROSPUN PATCH FOR ANTERIOR CRUCIATE LIGAMENT REPAIR

Edyta Augustyniak[P][1], Luka Savic[1], Adele Kristina Kastensson[2], Sarah Jane Bothwell Snelling[1], Pierre Alexis Mouthuy[1]

[1] *Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, OX3 7LD, United Kingdom.*

[2] *Department of Orthopedic Surgery, Karolinska University Hospital, SE-17176, Stockholm, Sweden.*

Corresponding author's email: edyta.augustyniak@NDORMS.ox.ac.uk

Failure rates of primary ACL reconstruction surgery approach 25%(1), necessitating new approach to improve outcomes. One promising strategy harnesses bioresorbable scaffolds to facilitate native ligament healing. Ideally, such scaffold should have a structure mimicking that of the natural ligament, provide adequate mechanical strength, and be fully biocompatible – supporting the appropriate cellular activity in order to drive tissue regeneration, while eliciting minimal local and systemic toxicity. With this in mind, we have developed a robust polycaprolactone(PCL) prototype scaffold, and have characterised it against a current market competitor as well as traditional suture materials. The prototype scaffold was produced by electrospinning of continuous PCL filaments(2), and by subsequently weaving these into a 10mm wide fabric. Uniaxial tensile testing was used to determine the mechanical properties of the prototype vs controls, and morphology was assessed via SEM. In compliance with ISO 10993-5:2009, cytotoxicity was evaluated with a neutral red uptake assay using NIH/3T3 cells. Cellular attachment and proliferation of ACL-derived primary fibroblasts, cultured on scaffolds for 14 days, were monitored using PrestoBlue™ assay and SEM. The tensile strength of the prototype patch was comparable to hamstring tendon allograft, the current gold standard for repair. The SEM images demonstrated a biomimetic morphology of the patch, with aligned nanofibers, similar to the ligament microstructure. The prototype showed an adequate cytotoxicity profile. Primary cells attached to the materials tested, demonstrating fibroblast-like morphology and increased metabolic activity from day 7 of culture. This study supports the suitability of a novel PCL electrospun scaffold for ACL reconstruction.

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Abstract **#670**

SELF-ASSEMBLED CONDUCTIVE NANOFIBERS FOR SPINAL CORD REGENERATION

Gillian Koehl[P][1], Josef Goding[1], Simone Di Giovanni[1], Rylie Green[1]

[1] *Imperial College London*

Corresponding author's email: gek13@ic.ac.uk

Spinal cord injury (SCI) is a severely debilitating chronic injury. Tissue repair remains a challenge due to the inability of severed axons to cross the injury site and form new connections. In vivo, injectable hydrogels have been shown to promote limited axonal growth through the lesion site, however they offer poor axonal guidance cues and connection to endogenous tissues. Cellular cues such as topography, mechanical properties, electrical stimulation and bioactive epitopes have shown promise in controlling neuroprogenitor differentiation, guidance, synapse formation as well as mediating inflammation. However, due to a lack of available materials, these cues have not been combined into an injectable aligned conductive fibre scaffold to foster regeneration after SCI.

Self-assembled peptides have shown promise as an injectable biocompatible hydrogel for SCI with aligned fibre topography [1]. Conductive polymers have shown potential for nerve tissue engineering applications due to their biocompatibility and conductivity. However, fabrication techniques have limited their incorporation into fibrous scaffolds to conductive coatings which limit cell adhesion and the incorporation of other bioactive cues.
In this work a pathway for the synthesis of a self-assembling amphiphilic peptide base VVAAEE functionalised with an alkyl tail and aniline monomer was designed. The effects on self assembly were assessed with circular dichroism and TEM. Future studies will investigate the trade-off between mechanical properties and conductivity, as well as the influence of fiber alignment on conductivity. The cellular response to this scaffold will also be investigated, through cytotoxicity studies, as well as differentiation and network formation.

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Abstract **#671**

PROXIMAL TUBULE INJURY PARACRINE FACTORS INDUCE THE DEPOSITION OF A COLLAGENEOUS EXTRACELLULAR MATRIX

Silvia M. Mihaila[1,2], João Faria$_P$[1], Laura Giordano[1], Rosalinde Masereeuw[1,2], Marta Valverde[3]

[1] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands

[2] Department of Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, The Netherlands

[3] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands

Corresponding author's email: j.p.ferreirafaria@uu.nl

Aim: Acute kidney injury is a pathological condition in which ischemia or drug-induced damage contribute to loss of proximal tubule cells. Stem cells therapies were shown to contribute in the repair process via paracrine mechanisms [1]. However, little is known about their contribution to the local extracellular matrix (ECM). With stem cells being a remarkable source of ECM, we hypothesized that their exposure to injured proximal tubule-derived paracrine factors will results in phenotypical and ECM composition changes. Methods: Proximal tubule injury was induced by exposure of human conditionally-immortalized proximal epithelial cells (ciPTEC-OAT1) for 24h to tenofovir (50µM) and antimycin-A (10nM). Post-injury secretome was screened for TGF-β/ PDGF-BB. Human adipose-derived stem cells (hASCs/P6) were exposed to ciPTEC-OAT1s-derived secretome (1:1, medium:secretome) enriched with ascorbic acid (50µg/mL). After 7 days of culture, protein quantification and deposition were evaluated. Results: Different proximal tubule cells-derived secretome compositions were obtained. While the exposure to tenofovir (50µM) reduced the metabolic activity ($p<0.001$), exposure to antimycin-A, known to inhibit mitochondrial oxygen consumption [2], did not affect the metabolic activity of ciPTEC-OAT1, when compared to standard cultures. Levels of TGF-β1 and PDGF-BB were significantly higher ($p<0.05$) in injured ciPTECs-OAT1s. A library of hASCs-made ECMs was obtained by the exposure to variable secretome compositions. The injury secretome (tenofovir- and AA-derived, respectively) induced higher protein deposition, enriched in collagenous proteins ($p<0.05$). Conclusion: Our results highlight stem cells as efficient producers of ECM, in the context of renal tubule repair. Further analysis includes in-depth evaluation of secretome, ECM and phenotypical changes.

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Abstract **#672**

BIOPRINTING OF A ZONAL-SPECIFIC CELL DENSITY SCAFFOLD: A BIOMIMETIC APPROACH FOR CARTILAGE TISSUE ENGINEERING

Pedro J. Diaz-Payno[P][1,2], Angeliki Dimaraki[1], Michelle Minneboo[1], Mohammad J. Mirzaali[1], Mahdiyeh Nouri-Goushki[1], Nicole Kops[2], Roberto Narcisi[2], Gerjo J.V.M. van Osch[1,2,3], Lidy E. Fratila-Apachitei[1], Amir A. Zadpoor[1]

[1] *Department of Biomechanical Engineering, Faculty of Mechanical, Maritime and Materials Engineering, Delft University of Technology, Delft, The Netherlands*

[2] *Department of Orthopaedics, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

[3] *Department of Otorhinolaryngology, Erasmus MC University Medical Center, Rotterdam, The Netherlands*

Corresponding author's email: p.j.diazpayno@tudelft.nl

Articular cartilage (AC) is a graded tissue with three layers exhibiting differences in cell density: the superficial zone having the highest and the deep zone, the lowest [1]. Only a few studies have introduced a cell gradient into scaffolds [2] and, as far as we are aware, none have optimized the scaffold mechanical properties, hence failing to recapitulate a more biomimetic environment. In this study, we aimed to bioprint scaffolds with different zonal cell densities, assess the scaffold stiffness and the influence of the cell density on tissue deposition.

The scaffolds were bioprinted using an alginate-based bioink (Cellink) containing human articular chondrocytes and a poly-caprolactone (PCL) support structure. The design of the scaffolds included three cell densities: 20E6 (high), 10E6 (mid) and 5E6 cells/ml (low). Eight different PCL designs were assessed by compressive tests. The scaffolds were cultured in chondrogenic medium for 25 days and analysed by live-dead and histological staining (Alcian blue, Hematoxilin-Eosin, and Picrosirius-red).

The PCL design showing the closest stiffness to native AC (8.35 ± 0.35 MPa) was used for bioprinting of the zonal scaffolds. The live-dead analysis at day 14 revealed the ability to generate a zonal cell density with high viability. Histology showed a smooth transition between the zones and a higher sGAG deposition in the highest cell density zone.

This qualitative data demonstrate the generation of different zonal cell densities within bioprinted scaffolds recapitulating the tri-phasic organization of AC. Follow-up studies should look at the addition of a stiffness gradient along the cell gradient.

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Abstract **#673**

ASSESSING THE FUNCTIONALITY OF BIOENGINEERED PROXIMAL TUBULES FOR BIOARTIFICIAL KIDNEY APPLICATIONS

Silvia M Mihaila[P][1,2], Dimitrios Stamatialis[3], Igor R Middel[1], Marianne C Verhaar[2], Karin GF Gerritsen[2], Rosalinde Masereeuw[1,2]

[1] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands

[2] Department of Nephrology and Hypertension, University Medical Center, The Netherlands

[3] (Bio)artificial organs, Department of Biomaterials Science and Technology, University of Twente, The Netherlands

Corresponding author's email: s.mihaila@uu.nl

Aim: Protein-bound uremic toxins (PBUTs) accumulate in plasma of end-stage kidney disease patients. Recently, we have developed bioengineered kidney tubules capable of active PBUTs secretion via organic anion transporter-1 (OAT1)[1]. To accelerate their application as units for bioartificial kidney (BAK), a comprehensive assessment of their functional performance under clinical-like conditions is essential. Here, we evaluated the PBUTs clearance capacity in bioengineered tubules perfused with uremic plasma and directly exposed to dialysate.

Methods: Human conditionally-immortalized proximal tubule epithelial cells equipped with OAT1 (ciPTEC-OAT1) [2] were exposed to medical-grade dialysate fluid for 240min. Their metabolic activity (PrestoBlue), membrane integrity (LDH release), pro-inflammatory response (IL-6/IL-8 release), oxidative stress (ROS production) and OAT1 functionality (fluorescein uptake) were assessed. Further, ciPTEC-OAT1s grown on biofunctionalized hollow fiber membranes were extraluminally exposed to dialysate fluid and intraluminally perfused with human uremic plasma using a tailor-made flow system. Clearance of five representative PBUTs was assessed (LC-MS/MS).

Results: Exposure of ciPTEC-OAT1s monolayers to dialysate reduced their metabolic activity (80.4±4.0%,$p<0.001$) and OAT1 functionality (80.9 5.4%,$p<0.001$) and increased the release of LDH (15.0±2.7 %,$p<0.05$), without inducing the release of IL-6 or IL-8. After 30min exposure, a 3.4±0.9-fold increase in ROS production was noticed. Furthermore, exposure of ciPTEC-OAT1s-loaded bioengineered kidney tubules to dialysate enabled the concomitant clearance of multiple PBUTs, without compromising the membrane integrity (FITC-inulin paracellular leakage).

Conclusion: The demonstrated functionality of bioengineered kidney tubules advances the development of BAK. Further research is aimed at introducing flow at the dialysate side and evaluating the efficacy of a multiple-fiber mini-BAK-module in rodents.

Keywords

bioartificial kidney; dialysis

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Abstract **#674**

DEVELOPMENT OF A HIGH-DENSITY SYSTEM FOR EXPANSION AND CARDIAC DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS AS AGGREGATES IN SINGLE-USE VERTICAL-WHEEL™ BIOREACTORS

Diogo E.S. Nogueira[P][1], Carlos A.V. Rodrigues[1], Marta S. Carvalho[1], Cláudia C. Miranda[1], Yas Hashimura[2], Sunghoon Jung[2], Brian Lee[2], Joaquim M.S. Cabral[1]

[1] Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

[2] PBS Biotech, Camarillo, CA, USA

Corresponding author's email: diogoespiritosantonogueira@tecnico.ulisboa.pt

Usage of human induced pluripotent stem cells (hiPSCs) for Regenerative Medicine applications requires their expansion to clinically-relevant quantities. The novel Vertical-Wheel bioreactors (VWBRs) allow for homogeneous mixing while conveying less shear stress to cells compared to traditional alternatives. This work reports strategies to establish and optimize expansion and cardiac differentiation of hiPSCs as aggregates in VWBRs [1].

Cultures were performed in the PBS MINI 0.1 bioreactor with 60 mL of working volume. Two different expansion media were tested, mTeSR1 and mTeSR3D, as well as dextran sulfate (DS) supplementation. The generated hiPSCs were analysed by flow cytometry and qRT-PCR and their differentiation potential was assayed via EB formation and directed differentiation. Cardiac differentiation was performed adapting a previously-described protocol [2]. A maximum cell density of (2.3±0.2)×10^6 cells·mL^–1 was obtained after 5 days with mTeSR1+DS, resulting in aggregates with an average diameter of 346±11 µm, without loss of pluripotency. Cardiac differentiation resulted in over 70% cTNT+ cells after 15 days.

The results here presented suggest the VWBR as a promising technology for the development of hiPSC-derived products under Good Manufacturing Practices for biomedical applications.

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Keywords

Human induced pluripotent stem cells; Aggregates; Cardiac differentiation

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Abstract **#675**

BOOSTING NEUROGENESIS: A NON-INVASIVE DEEP BRAIN STIMULATION PLATFORM FOR NEUROREGENERATION THERAPIES

Sofia Peressotti[1], Josef Goding[1], Rylie Green[1]
[1] *Imperial College London - Bioengineering Department*
Corresponding author's email: sp3915@ic.ac.uk

Alzheimer's disease (AD), among all neurodegenerative diseases, encompasses one of the most pressing healthcare crises to date. Due to the brain's intrinsically low turnover, neural tissue regeneration is considered an essential prerequisite to reverse AD cognitive and physical symptoms [1]. However, the development of a viable therapy is hindered by the low efficacy and immunogenicity of cell transplants as well as the inflammatory response due to invasive treatments. Electrical stimulation is known to increase neural stem cell (NSC) proliferation and differentiation [2]. Thus, a deep brain stimulation (DBS) method based on temporally interfering electric fields is hypothesised to direct NSC behaviour non-invasively [3]. The study aims to engineer a regenerative therapy to promote intrinsic adult neurogenesis and to improve the efficacy of NSC transplants through the use of biomimetic stimulation patterns compatible with non-invasive DBS. Electrical stimulation patterns mimic developmental brain waves and clinical DBS parameters, expecting to trigger neurogenesis-related molecular pathways. An autoclavable stimulation device is developed to simulate the non-invasive DBS clinical setting, and the effect of biomimetic stimulation on NSC fate and function in vitro is investigated in both 2D and 3D culture. The effect of stimulation on epigenetic regulation of cell differentiation, activation of developmental pathways, synapse formation and electrophysiological function is investigated. Overall, this minimally invasive approach embodies an unprecedented intersection between regeneration effectiveness and ease of clinical application, creating a possible pathway toward the treatment and reversal of neurodegeneration.

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Abstract **#676**

SOFT ROBOTIC DEVICES FOR EMULATING VASCULAR MECHANOBIOLOGY

Cody Fell[P][1], Mia Woodruff[1], Mark Allenby[1]

[1] *Queensland University of Technology*

Corresponding author's email: cody.alexander.fell@gmail.com

Tissue-engineered vascular grafts (TEVGs) are expected to replace prosthetic and autologous grafts as the gold standard for treating severe cases of vascular stenosis and occlusions in various formats, such as peripheral arterial disease (PAD), which affects over 200 million people worldwide (1). The femoropopliteal artery (FPA) accounts for nearly 80% of PAD cases and is one of the most flexible arteries in the body, accommodating standing, walking, and crouching (1, 2). These complex biomechanics and hemodynamic forces are implicated in the frequent incidence of PAD in the FPA, leading to debilitating morbidities, such as limb-threatening ischemia (3, 4). Moreover, FPA disease management and surgical treatments exhibit dismal long-term success rates, as low as 39% after five years (5). The emerging field of soft robotics aims to emulate physiological locomotion and could provide a platform for enhanced TEVG development and disease modelling applications that more completely mimic vascular biomechanics. We fabricated bio-integrated soft robotic devices (BSRs) imbedded with pneumatic networks (PneuNets) that induce angular flexion, longitudinal extension, and radial distension upon pressurization. We developed a coated hyperelastomer for both soft robotic actuation and tissue culture. Then we employed simulation-based iteration to design PneuNets optimized for emulating FPA flexion during daily walking and sitting poses, the most extreme being a radial distension of 20% and angular flexion of 140°. Finally, these designed, manufactured, and programmed vascular BSRs were seeded with mesenchymal stem cells (MSCs) and conditioned for 24 hours to highlight the effect of FPA mechanobiology on MSC cell morphology and alignment.

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Abstract **#679**

ALLOGENIC COLLAGEN DURAL SUBSTITUTE SUCH AS NOVEL ALTERNATIVE OF SYNTHETIC AND XENOGENIC DURAL GRAFT ON NEUROSURGICAL PROCEDURES.

Brenda Karen Aguillon-Estrada[P1], Grecia Andrea Cardoso Hernández[1], Eduardo Miramontes-Beas[1], Beni Camacho-Pérez[1], Juan Pablo Aguilar Alemán[2,1]

[1] *Top Health S.A.P.I de C.V.*

[2] *Tecnologico de Monterrey. School of Engineering and Science*

Corresponding author's email: greciacardo@hotmail.com

Due the common dural damage occurrance on neurosurgery, a proper closure of the site must be done in order to prevent complications on recovery [1]. Above fulfill tasks as provide watertight closure and support for cellular migration. Therefore, we developed and evaluated a formaldehyde crosslinking allogenic collagen sponge (ACS) as an alternative for the synthetic and xenogenic dural substitutes on neurosurgical procedures. Acid soluble collagen was extracted from human tissue pre-treated on alkaly solution and acid/enzymatic hydrolysis. The obtained solution was salting out for separated collagen type I fibers and homogenized on an acid solution. Thus, the excess of salt were removed by a dyalisis system. Finally, after concentrated the fibers with a freeze-drying process, the fibers were resolubilizated, lyophilizated on a specific shape and crosslinked with formaldehyde vapor treatment.The soluble collagen present the three characteristic α1, α2 and β bands for collagen type I on a SDS-PAGE electrophoresis gel [2,3]. An ACS of 1 cm2 had a complete in vitro degradation within 2-4 hours[4], a wide pore size range from 20 to 170 µm and below 10 ppm of residual formaldehyde [5]. Moreover, the crosslinking process did not affect the cytocompatibility; this was confirmed by seeding Human Adipose Stem Cells onto the ACS and stained by DAPI and Phalloidine after 14 days in culture. Finally, we observed that the ACS supported and promoted cell growth and migration and could be a possible scaffold for dura matter with possible low immune response due to allogenic source.

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Abstract **#681**

DESIGN OF A TISSUE-ENGINEERED 3D SPINAL CORD MODEL TO BETTER UNDERSTAND AMYOTROPHIC LATERAL SCLEROSIS

Aurélie Louit$_P$[1,2], Marie Josée Beaudet[1], François Berthod[1,2]

[1] *LOEX, Centre de recherche du CHU de Québec – Université Laval, Québec, Canada*

[2] *Département de Chirurgie, Faculté de Médecine, Université Laval, Québec, Canada*

Corresponding author's email: aurelie.louit.1@ulaval.ca

Background: A mutation in the Superoxide Dismutase 1 (SOD1) gene has been identified as a cause of amyotrophic lateral sclerosis (ALS) in 5% of patients. Recently, studies have shown that non-neuronal cells could contribute to the development of ALS. Objectives: Our objectives are to develop by tissue engineering an in vitro murine 3D model of spinal cord reproducing the ALS phenotype, and to determine the role of each cell type in its development. Methods: Motor neurons (MN) have been extracted from spinal cord mouse embryos aged of 14 days of development and astrocytes, microglia, and Schwann cells (SC) from SOD1G93A or SOD1WT adult mice, overexpressing respectively the ALS mutant or the normal human SOD1 protein. These cells have been purified and co-cultured on 3D collagen/chitosan sponges. Results: When SOD1G93A MN were cultured with mutant astrocytes and microglia, there was a 25% reduction in TUJ-1 positive neurites length, compared to the control seeded with SOD1WT MN, astrocytes and microglia. A similar decrease in the neurite length was observed with non-transgenic MN grown in presence of SOD1G93A glial cells, compared to SOD1WT glial cells. Finally, when cultured with SOD1WT SC, axonal growth was enhanced. Conclusion: Axonal migration was found shorter in presence of SOD1G93A glial cells, recapitulating in part an ALS phenotype. However, adding SC to the model helps axonal growth. This 3D model, adaptable to other types of mutations involved in ALS, should lead to a better understanding of the disease mechanisms, and could serve as a platform for drug screening.

Keywords

Amyotrophic lateral sclerosis; Motor neuron disease; In vitro 3D model

Abstract **#682**

INJECTABLE POROUS DOUBLE-NETWORK HYDROGELS WITH VOCAL FOLD-MIMETIC VISCOELASTICITY

Fatemeh Taheri$_P$[1], Guangyu Bao[2], Sepideh Mohammadi[2], Luc Mongeau[2]

[1] *McGill University, Montreal, QC, Canada; Centre for Interdisciplinary Research in Music Media and Technology (CIRMMT)*

[2] *Department of Mechanical Engineering, McGill University, Montreal, QC, Canada*

Corresponding author's email: fatemeh.taheri@mail.mcgill.ca

Healthy voice production has a significant impact on individual wellness, occupational function, and societal productivity. Intense mechanical stresses during speech can result in laryngeal dysfunctions, including vocal folds (VFs) scarring and tissue loss. The most common treatment in practice is to inject biomaterials to the wounded site; however, the mechanical properties of existing biomaterials for VF treatments are poorly matched to those of native VFs or they could be effective over only a short time period. We investigated a chitosan-based biocompatible and injectable double-network hydrogels to recapitulate both the structural and viscoelastic properties of VFs. Our porous double-network hydrogels comprise 1-2 % glyoxal-crosslinked glycol-chitosan (covalent network) and 1.5% physically crosslinked chitosan (sacrificial network). During gelation, the chitosan network exhibited pH-induced phase separation, resulting in the creation of micropores. We used confocal microscopy and micro-CT to characterize the pore size of the hydrated gels and found them to be within 10-20 µm. By varying the glycol-chitosan concentration, the stiffness (1-3 kPa) and stress relaxation time (20-120 seconds) were highly tunable to match those of VFs. The obtained relaxation time is significantly faster than that of most covalently crosslinked hydrogels (>10,000 seconds). This biomimetic viscoelasticity is attributed to the breaking of dissipative physical crosslinks and highly interconnected pores to facilitate water migration under strain. These hydrogels are also friendly to human vocal fold fibroblasts, yielding over 75% cell viability rates over a 7-day 3D fibroblast culture. The efficacy of this hydrogel system will be evaluated using a phono-mimetic bioreactor.

Keywords

Vocal Fold hydrogel; viscoelastic properties; porosity

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Abstract **#683**

PRECLINICAL EVALUATION OF CELLULAR NANOMESHES FOR VAGINAL REPAIR IN AN OVINE MODEL OF PELVIC ORGAN PROLAPSE

Shayanti Mukherjee$_P$[1,2], Saeedeh Darzi[1], Kallyanashis Paul[1,2], Permita Mazumder[1], Anna Rosamilia[3,2], Helmut Thiessan[4], Jerome Werkmeister[1,2], Caroline Gargett[1,2]

[1] *The Ritchie Centre, Hudson Institute of Medical Research, Clayton VIC Australia 3168*

[2] *Department of Obstetrics and Gynaecology, Monash University, Clayton VIC Australia 3168*

[3] *Pelvic Floor Disorders Unit, Monash Health Australia VIC 3168*

[4] *CSIRO Manufacturing,Research Way, Clayton VIC Australia 3168*

Corresponding author's email: shayanti.mukherjee@hudson.org.au

Pelvic Organ Prolapse impacts 1 in 4 women, due to weakening of vaginal wall due to childbirth [1]. Non-degradable synthetic mesh for treating pelvic organ prolapse (POP) have been banned in many countries due to the adverse events associated with transvaginal surgery [2]. To this end, we developed poly (L-lactic acid)-co-poly(ε-caprolactone) electrospun nanofibrous mesh bioengineered with SUSD2+ endometrial mesenchymal stem/stromal cells (eMSC) for POP repair. This study determines the impact and fate of our vaginal therapy with focus on tissue integration and foreign body response using our unique ovine model of POP (n=45). We compared our results to NanoMesh without eMSC and to current clinical native tissue repair procedure as a control. Following surgery, vaginal tissues were explanted and assessed at 7, 30 and 90 days. Firstly, we observed that surface modification of meshes had a significant impact on the foreign body response and giant cell formation. Surface modified nanomeshes with and without eMSCs integrated well with vaginal tissues revealing formation of new extracellular matrix (ECM) and blood vessels inside the meshes, confirmed by histology and scanning electron microscopy. We are currently assessing macrophage polarization, inflammation, elastin metabolism as well as tensile properties from vaginal explants from sheep. Our study highlights that eMSCs modulate immune response and thus impact the fate of implants used for POP treatment. From a tissue engineering perspective, such bioengineered constructs have a significant potential as alternative autologous surgical constructs for the treatment of POP.

Keywords

gynecology; nanofiber; immune response

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Abstract **#684**

MATRIX-BOUND NANOVESICLE-ASSOCIATED IL-33 MODULATES MACROPHAGE PHENOTYPE TRANSITION IN THE HOST RESPONSE TO SKELETAL MUSCLE INJURY

Arthi Shridhar[P][1,2], Joseph Bartolacci[3,2], Marissa Behun[3,2], Amrita Sahu[2], Gaelen Dwyer[1], Fabrisia Ambrosio[2,4], Heth Turnquist[1], Stephen Badylak[1,2]

[1] *University of Pittsburgh, School of Medicine*

[2] *McGowan Institute for Regenerative Medicine*

[3] *University of Pittsburgh, Department of BioEngineering*

[4] *University of Pittsburgh, Department of Physical Medicine & Rehabilitation*

Corresponding author's email: ars307@pitt.edu

Favourable tissue remodelling requires an appropriately-timed transition from a pro-inflammatory (M1-like) to a pro-remodelling (M2-like) macrophage response. Matrix bound nanovesicles (MBV) activate macrophages toward an M2-like phenotype, and are a rich source of interleukin-33 (IL-33). IL-33 can attenuate inflammation, and enhances satellite cell expansion for muscle regeneration. This study evaluated the effects of MBV-associated IL33 on macrophage phenotype and associated muscle regeneration. Bone marrow macrophages and MBV were isolated from C5BL/6 and C5BL/6-il33-/- (IL33 KO) mice1. Muscle stem cells (MuSC) were isolated from wild-type mice, and treated with (i) recombinant IL33, (ii) IL33-MBV or (iii) IL33+ MBV for 4 d (1 x 109 MBV/mL). Additional groups included supernatants from macrophages treated with the same. MuSC differentiation was evaluated through immunolabeling for myosin heavy chain. For in vivo studies, a muscle injury was induced using cardiotoxin in (IL-33 KO) mice2 and IL33+ MBV was delivered to the tibialis anterior. At 3, 7 and 14 d, cells were immunolabelled for M1-like (Cd11b/iNOS), M2-like (Cd11b/Fizz) macrophages, and satellite cells (MyoD). Muscle function was evaluated using in situ contractile testing at 14 d. Treatment with exclusively IL33+MBV promoted an M2-like macrophage phenotype, and enhanced myogenesis. The latter was observed solely in the indirect treatment group, suggesting a possible macrophage-mediated mechanism. Impaired functional recovery and reduced satellite cell activation in IL33 KO mice, was partially restored upon exogenous provision of IL33+ MBV. MBV-associated IL-33 plays a significant role in promoting a macrophage transition that is conducive for the functional repair of muscle following injury.

Keywords

Immunomodulatory ; Matrix bound nanovesicles; Interleukin-33

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Abstract **#685**

TISSUE ENGINEERED HYDROGEL BLOCKS AS A MODEL TO STUDY BONE-TENDON INTERFACE DEVELOPMENT

Ilze Donderwinkel$_P$[1], Rocky S Tuan[2], Neil R Cameron[1,3], Jessica E Frith[1]

[1] *Monash University*

[2] *Chinese University of Hong Kong*

[3] *University of Warwick*

Corresponding author's email: ilze.donderwinkel@monash.edu

Tendon injuries account for the highest percentage of musculoskeletal clinical complaints [1]. Due to the replacement of the native structure by scar tissue the bone-tendon interface (enthesis) weakens, and full function is rarely restored. Tissue engineering is a promising strategy to increase understanding of enthesis formation and repair, a requirement of functional enthesis regeneration. In this study, we developed a tissue-engineered model with which to study enthesis development based upon the encapsulation of human bone marrow mesenchymal stromal cells (hBMSCs) in a biocompatible hydrogel.

Hydrogels were formed by 400-500 nm blue light photocrosslinking of methacrylated gelatin and a poly(D,L-lactic acid) and poly(ethylene glycol) co-polymer. Rheometry revealed a storage modulus of 1.93 ± 0.23 kPa with hydrogels showing a degradation time of >48 days. Biocompatibility was confirmed with encapsulated hBMSCs showing high cell viability (>80%), cell spreading (actin staining) and proliferation (Ki67 staining). We then formed separate blocks of tissue-engineered tendon and bone to study enthesis development at the tissue interface. Upregulation of tenogenic markers and tendon-like tissue formation showed optimized tenogenic differentiation under TGF-β3 and mechanical loading. High mineralization indicated osteogenic differentiation of encapsulated hBMSCs. This hydrogel platform was then used to study the influence of paracrine signalling on artificial enthesis formation.

Together, our findings support the utility of our system as a tissue engineering platform to study enthesis formation and development. This system enables further analysis of the influence of paracrine signalling at the enthesis and the mechanisms responsible for artificial enthesis development.

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Abstract **#686**

CUTANEOUS WOUND HEALING CAN BE ENHANCED THROUGH THE APPLICATION OF TYPE I COLLAGEN HYDROGELS MODIFIED WITH OLIGOURETHANE AND SILICA

Pedro Ulises Munoz-Gonzalez[P][1,2], Maria Cruz Lona-Ramos[2], Gerardo Gonzalez-Garcia[1], Jorge Delgado[2], Laura Castellano[2], Birzabith Mendoza-Novelo[2]

[1] *División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Guanajuato, Mexico.*

[2] *División de Ciencias e Ingenierías, Universidad de Guanajuato, León, México.*

Corresponding author's email: pu.munozgonzalez@ugto.mx

Cutaneous wound healing process leads to the formation of scar tissue rather than the restoration of functional and aesthetically satisfactory skin. Wound healing process consists in three precisely and highly programmed phases: inflammation, proliferation, and remodeling. In this work, a composite collagen hydrogel with tunable properties is proposed as a candidate for holding and releasing components intended to heal skin wounds. Type I collagen hydrogels were crosslinked with oligourethane and loaded with colloidal silica, and optionally, with anti-infection agents. The in vitro macrophages response to hydrogel was studied, as well as the physicochemical and mechanical properties. Composite collagen hydrogels were deposited in situ on the rat skin wounds and the formed skin was examined histologically. Hydrogels demonstrated to be suitable for injection reaching storage modulus around 500Pa after a 15min of gelation. The collagen crosslinking modulated in vitro degradation and water uptake capacity and did not prevent the viability of dental pulp stem cells and macrophages. Macrophages cultured within hydrogels produced IL-1β, IL-10 and TGF-β1; commonly associated with an immunoregulation response. This silica-doped crosslinked collagen injectable hydrogel accelerated wound contraction, reaching 90% in 10 days, compared to the 75% of controls. Wound healing was featured by a shorter inflammatory stage and accelerated angiogenesis, fibroblast ordering, and dermal maturation after composite hydrogel application. Altogether these results suggest that hydrogel components act synergistically to promote functional wound closure, suggesting a role in the inflammatory and proliferation phases.

Keywords

Crosslinked collagen hydrogels doped with silica; Dermal wound healing biomaterials; Inflammation modulation

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Abstract **#690**

CELL SPHEROID CULTURE SYSTEM USING POROUS MICROPARTICLES FOR ENHANCED CELL SURVIVAL AND INDUCE BONE REGENERATION

Min Ji Kim[P][1], Yeong Jo Jeoung[2], Jin Ho Lee[2], Se Heang Oh[1]

[1] *Department of Nanobiomedical Science, Dankook University, Cheonan 31116, Korea*

[2] *Department of Advanced Materials, Hannam University, Daejeon 34054, Korea*

Corresponding author's email: seheangoh@dankook.ac.kr

In recent years, a cell spheroid system is considered as one of the most common and versatile way to culture cells in 3D. However, limited size, weak structural stability and heterogeneous cell differentiation of cell spheroid due to the insufficient supply of oxygen/nutrients into the central region, relatively weak cell-cell interaction, and different concentration of signaling molecules along the cell depth of the spheroid are still remained as critical challenges. In this study, we fabricated porous microparticles with leaf-stacked structure throughout entire matrix (ELSS particles) which can provide space for sufficient supply of oxygen/nutrients in cell spheroids, act as a filler for improved structural stability of cell spheroids, and allow sustained release of signaling molecules for effective induction of cell differentiation throughout whole cell spheroids. A cell spheroid system was prepared using the ELSS particles and human bone marrow-derived mesenchymal stem cells (hBMSCs). The morphology, release pattern of bone morphogenetic protein-2 (BMP-2) from ELSS particles, cell survival/structural stability/osteogenic differentiation of hBMSCs in cell/ELSS spheroid system were observed. We also evaluated cell/ELSS spheroid system for promoting bone regeneration in a SD rat (calvarial defect model).

Acknowledgments

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Keywords

Cell spheroid; Polycaprolantone; Bone regeneration

Abstract **#691**

ADJUSTABLE CONDUITS FOR GUIDED PERIPHERAL NERVE REGENERATION PREPARED FROM BI-ZONAL UNIDIRECTIONAL AND MULTIDIRECTIONAL LAMINAR SCAFFOLD OF TYPE I COLLAGEN

Marta Fontanilla[P][1], Diana Millán[1,2], Ronald Jiménez[1,3], João Mano[4], Ana Silva[4]

[1] *Tissue Engineering Group, Department of Pharmacy, Universidad Nacional de Colombia*

[2] *Universidad El Bosque, Facultad de Medicina*

[3] *BUniversidad El Bosque, Facultad de Ciencias*

[4] *Department of Chemistry, CICECO, University of Aveiro.*

Corresponding author's email: dmmillanc@unal.edu.co

Shortness of donor nerves for grafting peripheral nerve injuries has led to the development of nerve guidance conduits that connect sectioned stumps and thus help to prevent the formation of neuromas. Often, the standard diameters of these devices cannot be adapted to the diameter of the nerve injured at the time of surgery. In the present work, bi-zonal scaffolds were developed to form nerve conduits filled with an inner matrix made of unidirectional channels, which is covered by a multidirectional pore zone. Collagen type I dispersions (5 mg/g and 8 mg/g) were sequentially frozen using different methods to obtain six laminar scaffolds. Each of these scaffolds consisted of a unidirectional pore/channel zone adjacent to a multidirectional pore zone. Conduits with adjustable diameter were formed by rolling-up the scaffolds from the unidirectional to the multidirectional zone. Physicochemical, microstructural, and mechanical properties of the scaffolds, as well as their biodegradability, residual glutaraldehyde, and cytocompatibility were determined and compared. Based on the results of these comparisons, proliferation and differentiation of human adipose-derived stem cells were assessed in only one of the scaffolds. These cells adhered, aligned in the same direction as the unidirectional pore fibers, proliferated, and differentiated into Schwann-like cells. The results of this work highlight the potential ability of the tested scaffold to form adjustable nerve guidance conduits capable of interacting with cells in vivo when grafted onto a severed nerve.

Keywords

Bi-zonal scaffolds ; Collagen type I; Peripheral nerve regeneration

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Abstract **#693**

DECELLULARIZATION OF 3D BIOPRINTED TISSUES TO ENGINEER 'OFF-THE-SHELF' IMPLANTS FOR BONE REGENERATION

Pierluca Pitacco[P][1,2], Daniel J Kelly[1,2,3]

[1] *Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland*

[2] *Department of Mechanical, Manufacturing and Biomedical Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland*

[3] *Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland*

Corresponding author's email: ppitacco@tcd.ie

Decellularization of the ECM deposited by cells in vitro represents a promising strategy to develop bioactive biomaterials as its composition can be engineered to meet specific requirements. The capacity to generate patient-specific implants can be further enabled by emerging 3D bioprinting strategies. The goal of this study was to generate engineered-ECM scaffolds for bone regeneration by decellularizing 3D bioprinted tissues primed in vitro for endochondral bone formation.

A fibrinogen-based bioink [1] containing hMSCs was 3D bioprinted within a polycaprolactone scaffold generated by fused deposition modelling to create mechanically reinforced constructs, which were subsequently cultured within one of three different culture regimes (chondrogenic, hypertrophic, chondrogenic+BMP-2) and then decellularized using freeze/thaw cycles, a wash in Triton X-100 + NH4OH and DNase treatment. Biochemical assays and histological analyses were performed pre- and post-decellularization, and after reseeded osteogenic culture.

After decellularization, DNA content dropped for all the regimes to less than 35% of the pre-decellularization value. sGAG levels decreased, but constructs retained 60% or more of the initial values, while approximately 80% of the deposited collagen was always retained. Compared to the uncultured bioink controls, all the decellularized implants better supported the osteogenesis of reseeded hMSCs under minimal osteogenic culture in vitro, with cells depositing the highest values of calcium on the hypertrophic constructs. A subcutaneous implantation pilot study in mice showed that decellularized grafts supported robust vascularization in vivo.

An ongoing rat femoral defect study is now exploring the potential of these three promising decellularized engineered-ECMs to promote bone regeneration.

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Abstract **#694**

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO CARDIOMYOCYTES ON CELL SORTING THERMORESPONSIVE SURFACE

Tzu Cheng Sung$_P$[1], Huan Chiao Su[2], Akon Higuchi[1,2,3]

[1] *School of Ophthalmology and Optometry, The Eye Hospital of Wenzhou Medical University*

[2] *Department of Chemical and Materials Engineering, National Central University*

[3] *Nano Medical Engineering Laboratory, RIKEN*

Corresponding author's email: szc@eye.ac.cn

The human pluripotent stem cells (hPSCs) are a stable and limitless source of regenerative medicine due to hPSCs differentiation into all types of cells in our body. However, current differentiation process of human pluripotent stem cells into cardiomyocytes to enhance the purity of hPSC-derived cardiomyocytes requires some purification processes, such as Fluorescence-activated cell sorting (FACS) or Magnetic-activated cell sorting (MACS), which are laborious and expensive. In this study, we developed cell sorting plates, which were prepared from coating thermoresponsive poly(N-isopropylacrylamide) and extracellular matrix proteins on the cell culture plates. After hPSCs were induced into cardiomyocytes on the thermoresponsive surface coated with laminin-521 for 15 days, the temperature of the cell culture plates was decreased to 8-9 ℃ to detach the cells partially from the thermoresponsive surface. The detached cells exhibited a higher cardiomyocyte marker of cTnT than the remaining cells on the thermoresponsive surface as well as the cardiomyocytes after purification using conventional cell selection. The detached cells expressed several cardiomyocyte markers, such as α-actinin, MLC2a, and NKX2.5. This study suggests that the purification of hPSC-derived cardiomyocytes using cell sorting plates with the thermoresponsive surface is a promising method for the purification of hPSC-derived cardiomyocytes with inexpensive and easy operational way compared to conventional laborious purification processes.

Keywords

human pluripotent stem cells; Cardiomyocyte; Cell sorting dish

Abstract **#695**

AN INJECTABLE FAMILY OF SOFT TISSUE HYDROGEL ADHESIVES FOR KNEE APPLICATIONS

Peyman Karami$_P$[1], Céline Wyss[2], Pierre-Etienne Bourban[2], Philip Procter[3], Christophe Moser[4], Dominique Pioletti[1]

[1] *Laboratory of Biomechanical Orthopedics, EPFL, Lausanne, Switzerland*

[2] *Laboratory for Processing of Advanced Composites, EPFL, Lausanne, Switzerland*

[3] *Applied Materials Science, Department of Engineering Sciences, Uppsala University, Sweden*

[4] *Laboratory of Applied Photonics Devices, EPFL, Lausanne, Switzerland*

Corresponding author's email: peyman.karami@epfl.ch

While various types of injectable tissue adhesives have been developed, insufficient adhesion performance of these materials to load-bearing soft tissues, as well as their poor physiochemical properties remain a challenge in biomedical applications. Commercially available adhesives such as fibrin- and PEG-based adhesives present low adhesion, and cyanoacrylate adhesives could cause cytotoxicity. Moreover, most developed hydrogel adhesives impose limitations such as non-injectability, high polymer content, low mechanical properties, slow implementation and the need for the host tissue surface treatment.

By controlling the interfacial bondings and bulk mechanics, we propose an intrinsically-adhesive design platform for the development of hydrogels with multifaceted functionalities. Our single network (SN) hydrogels were fabricated with (a) a two-step modification of a polymeric backbone (e.g., hyaluronic acid, gelatin), including methacrylation of the chains and subsequent chemical conjugation of adhesive molecules, and (b) incorporation of a reinforcing component (e.g., fibrillar collagen, fibrillated cellulose). With the versatility of the design strategy, not only a high level of adhesion under a very fast curing time was achieved, but wide window of physicochemical properties was obtained for the target applications. The potent adhesion performance of our hydrogels was studied by ex vivo and in vivo (goat model) evaluations.

We have particularly targeted the treatment of meniscus tears with the developed adhesive hydrogel as an unmet clinical need. Current surgical techniques involve either highly invasive and complex suturing methods or permanent removal of the damaged regions. Our findings highlight the potential of hydrogel family for minimally-invasive treatment of the meniscus tears.

Keywords

Regenerative Medicine; Tissue Adhesive; Translational Study

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Abstract **#697**

VAGINAL DELIVERY OF ADULT STEM CELLS IN ALOE VERA-ALGINATE HYDROGEL ALLEVIATES CHILDBIRTH TRAUMA IN A RAT MODEL

Kallyanashis Paul[P1,2], Saeedeh Darzi[2], Mark P Del Borgo[3], Fiona L Cousins[2,1], Jerome A Werkmeister[2,1], Caroline E Gargett[2,1], Shayanti Mukherjee[2,1]

[1] *Department of Obstetrics and Gynaecology, Monash University, Clayton Australia 3168*

[2] *The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia 3168*

[3] *Department of Pharmacology, Monash University, Clayton, Australia 3168*

Corresponding author's email: kallyanashis.paul@monash.edu

Vaginal childbirth causes significant maternal tissue injury, which can later lead to incurable disorders such as pelvic organ prolapse (POP). This study introduces an immediate vaginal tissue treatment using Aloe Vera (AV)-Alginate (ALG) hydrogel (Hyd) for delivering maternal endometrium derived Mesenchymal stem cells (eMSCs) to promote early healing in a rat simulated birth injury (SBI) model. Vaginal trauma after SBI was evidenced by increased vaginal diameter and inflammatory response. Untreated tissues showed a significant reduction of smooth muscle content, increased elastin and increased tissue stiffness, indicative of fibrotic healing. Local injection with hydrogel (Hyd T) or with eMSC in hydrogel (Hyd/eMSC T) had a significant impact on birth injury reversal. Hyd/eMSC T significantly improvement smooth muscle and elastin content, comparable to uninjured control vagina. At the nanoscopic level, injury caused disorganised structural collagen with increased D period but intervention with Hyd/eMSC T, normalised collagen structure and significantly reduced tissue stiffness. Hyd/eMSC injection showed significant immunomodulation evidenced by lowered M1:M2 ratio while restoring connective tissue composition after 6-weeks. Immediate treatment of severe vaginal birth trauma with therapeutic eMSCs delivered in AV-ALG hydrogel may be a potential new treatment strategy for healing birth injury and preventing future POP in women.

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Abstract **#698**

BIOACTIVE AND PHOTOLUMINESCENT ORGANIC-INORGANIC HYBRID MICROPARTICLES FOR BONE REGENERATION AND DUAL DRUG DELIVERY

Neda Aslankoohi$_P$[1], Kibret Mequanint[1,2]

[1] *School of Biomedical Engineering, Western University, London, Ontario, Canada*

[2] *Department of Chemical and Biochemical Engineering, Western University, London, Ontario, Canada*

Corresponding author's email: naslanko@uwo.ca

Introduction: Any material intended for bone tissue engineering requires having various properties in a single structure. This can be achieved by combining organic and inorganic materials with considerably different properties in the form of hybrids that retain the intrinsic properties of their constituents and acquire new, synergistic properties. Here, we have prepared spherical photoluminescent hybrid microparticles (MPs) from L-phenylalanine-derivatized poly(ester amide) (PEA) and tertiary bioactive glass (BG) and loaded them with two compounds.

Methods: Hybrid microparticles were synthesized using sol-gel process in the presence of PEA, with catalytic amounts of acid in a two-pot process. By adding Nile Blue and Orange G as hydrophobic and hydrophilic model compounds during synthesis, single- or dual-loaded MPs were obtained.

Results: Single-phase homogeneous hybrid microparticles were synthesized from PEA and BG. A nucleation-aggregation mechanism is proposed for the formation of the MPs. The homogeneity of the MPs was proven via EDX elemental mapping, while deposition of a layer of hydroxyapatite on the surface of MPs after a one-day incubation in simulated body fluid demonstrated their bioactivity. The MPs possess intrinsic photoluminescence properties owing to PEA. Nile Blue showed a sustained release profile for more than 14 days. The cytocompatibility of the MPs was demonstrated through culturing of mesenchymal stem cells embedded in a fibrin gel containing MPs for 2 weeks.

Conclusion: The bioactivity in cooccurrence with intrinsic photoluminescence and the possibility of dual drug release, all in a homogenous microparticle, offer a unique platform for bone tissue engineering, including both regenerative and pharmaceutical therapy.

Keywords

Organic-inorganic hybrid microparticles; Dual drug delivery; Bone tissue engineering

Abstract **#699**

GEOMETRY AND PORE SIZE OF MELT ELECTROWRITTEN MESHES DIRECT MACROPHAGE-MEDIATED FOREIGN BODY RESPONSE IN VIVO: IMPLICATIONS IN UROGYNECOLOGY

Kallyanashis Paul[P][1,2], Saeedeh Darzi[1], Jerome A Werkmeister[1,2], Caroline E Gargett[1,2], Shayanti Mukherjee[1,2]

[1] *The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Australia 3168*

[2] *Department of Obstetrics and Gynaecology, Monash University, Clayton Australia 3168*

Corresponding author's email: kallyanashis.paul@monash.edu

Pelvic organ prolapse (POP) impacts 65% of women over 68 years of age. Recently non-degradable transvaginal meshes were banned owing to the chronic inflammation and foreign body reaction. This study proposes to overcome such clinical hurdles by designing optimal degradable 3D printed melt electrowritten (MEW) meshes as a promising alternative, that can minimize undesirable immune response. Meshes of MEW poly ε-caprolactone (PCL) with hierarchical geometry were fabricated by two-way stacking of the strands at spacing: 1 mm, 0.5 mm at 3 different interlayer angle of 90, 45 and 22.5 Deg. The mesh morphology study by electron microscopy reveals the strand thickness to be 18.86 ± 2.16 µm. The hierarchical mesh printed at 45 and 22.5 Deg showed better proliferation of vaginal fibroblasts and tensile strength at the dry condition. The meshes were implanted in a mouse model of POP to assess the foreign body reaction at 1 and 6 weeks. Explants revealed hierarchical 45 and 22.5 Deg angular meshes showed better tissue integration. We are currently assessing the expression of CCR7 and CD206 in tissues to understand the impact of geometry on macrophage polarization. This study profiles the immunobiology following MEW mesh implantation to provide an optimal alternative in urogynecological surgery.

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Abstract **#700**

THE EFFECT OF PRIMARY ALLOGENEIC AND XENOGENEIC TENOCYTE DERIVED EXOSOMES ON MACROPHAGE DIFFERENTIATION OF THP-1 CELLS: AN APPROACH TO IMPROVE TENDON HEALING

Devin von Stade[P][1], Alissa Mathias[1], Jimmy Johnson[1], Daniel Regan[1], Kirk McGilvray[1]

[1] *Colorado State University*

Corresponding author's email: vonstade@colostate.edu

Extra cellular vesicles (EVs) are a primary paracrine effector of cell mediated improvements in tissue healing. The mechanism underlying this therapeutic benefit is thought to result indirectly from the immunomodulatory abilities of EVs, via triggering the secretion of cytokines that promote alternatively-activated (M2) anti-inflammatory macrophages. Our hypothesis is that EVs can eventually be functionally incorporated into 3D printed scaffolds to direct macrophage polarization, improving the tendon healing landscape. Thus, the goal of this study was to determine the effectiveness of cell specific EVs to direct monocyte / macrophage polarization into an M2 phenotype. EVs were purified via size-exclusion filtration from enriched media of primary tenocytes from human and ovine rotator cuff tendons harvested 2-15 hours post-mortem. Human monocytes (THP-1 cells) were differentiated into a macrophage phenotype using phorbol 12-myristate 13-acetate (PMA) and co-cultured with: 1) 100ug/mL purified exosomes, 2) exosome depleted tenocyte enriched media, 3) fetal bovine serum, or 4) exosome depleted fetal bovine serum. Cultures were analyzed using an immunologic multiplex assay examining IL-6, 1L-12, and IL-10 macrophage markers. Cultures challenged with human tenocyte derived exosomes showed marked upregulation of IL-10 expression, a primary M2 phenotype indicator, in relation to pro-inflammatory macrophage markers including IL-6 and IL-12 as compared to all other culture groups. These data showed a significant alteration in macrophage signaling toward an M2 phenotype for this specific exosome cohort. Future work will strive to incorporate EVs into 3D printed scaffolds as an approach to improve the surgical repair of rotator cuffs.

Abstract **#701**

ENDOMETRIAL MESENCHYMAL STEM CELLS MODULATE THE FOREIGN BODY RESPONSE TO ELECTROSPUN DEGRADABLE NANOFIBRES

Saeedeh Darzi[P][1], Janet Alappadan[2], Caroline E Gargett[1,2], Jerome A Werkmeister[1,2], Shayanti Mukherjee[1,2]

[1] *The Ritchie Centre, Hudson Institute of Medical Research*

[2] *Department of Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia*

Corresponding author's email: saeedeh.darzi@hudson.org.au

Pelvic Organ Prolapse (POP) is the herniation of pelvic organs into the vagina. Despite broad acceptance of mesh use in POP surgical repair, the complication rate is unacceptable. Tissue engineering techniques involving a biologic or synthetic scaffold seeded with regenerative and immunomodulatory cells could provide a promising alternative. This study aimed to determine the Foreign Body Response (FBR) to nanofibrous degradable poly (L-lactic-acid)-co-poly(e-caprolactone) blended with gelatin mesh (PLACL/G) seeded with endometrial mesenchymal stem cells (eMSC) implanted in a mouse model of wound repair and tissue regeneration.

eMSC were isolated from endometrial biopsy by SUSD2 magnetic bead sorting. Electrospun PLACL/G nano-meshes with and without eMSC were implanted in a NSG mouse skin wound repair model for 1 and 6 weeks (n=7 mice/group). The expression of extracellular matrix (ECM), cell adhesion, angiogenesis and immune response genes were quantified using Fluidigm Biomark qPCR.

Our results show significant changes in ECM, angiogenesis and immune response genes in the presence of eMSC. We found a significantly reduced expression of the inflammatory gene Il-6 in eMSC seeded PLACL/G group compared to PLACL/G alone after one week ($P< 0.05$). The expression of ECM associated genes including Col-III, Cd44 and Cdh2 increased from one to 6 weeks in the PLACL/eMSC group. Constructs including eMSC showed an increased expression of angiogenesis associated genes including Pdgfa at 6 weeks ($P<0.05$).

This study shows that eMSC modulate the FBR to degradable PLACL/G nanofibre mesh through down-regulation of acute inflammatory markers and improved tissue regeneration through induction of ECM synthesis and vascularization.

Keywords

Electrospun nanomesh; Foreign body response; mesenchymal stem cells

Abstract **#702**

CONVERGING MELT ELECTROWRITING WITH HIGH-THROUGHPUT 3D-BIOASSEMBLY TECHNOLOGIES

Gabriella Lindberg$_P$[1], Ezgi Bakirci[2], Gary Hooper[1], Khoon Lim[1], Paul Dalton[2], Tim Woodfield[1]
[1] *Christchurch Regenerative Medicine and Tissue Engineering Group, Uni. of Otago, New Zealand.*
[2] *Department for Functional Materials in Medicine and Dentistry, Uni. of Würzburg, Germany*
Corresponding author's email: Gabriella.Lindberg@otago.ac.nz

Recent work highlights the potential of seeding spheroids in high resolution melt electrowritten(MEW) scaffolds to generate larger hybrid constructs with mechanical support. However, the structure to function relationships of these constructs are poorly understood. This study aimed to develop a hybrid MEW 3Dbioassembly platform to study interfaces in high throughput. We systematically investigated the effect of fibre thickness, spacing and hydrophilicity of MEW scaffolds on spheroid seeding efficiency, stability as well as cell-material, and cell-cell interactions.

Allylated gelatin (GelAGE:G1MM,20wt%) and thiolated heparin (HepSH,0.5wt%)[1,2] was biofabricated into cell-laden microspheres(15x10ˆ6 hACs/ml, 5x10ˆ6 hMSCs/ml) using oil-emulsion microfluidics[3] and photopolymerisation(450nm,30mW/cm2,3min,1/10mM Ru/SPS,60mM DTT). MEW scaffolds were 3Dprinted[4,5] with 5µm catching-fibres and tailored fibre diameter (5, 10, 50µm), +/-NaOH treatment. 3Dbioassembled MEW-constructs were cultured in chondrogenic (5w) or osteogenic medium (3w). Cellular health (live/dead®/alamarBlue®) and tissue formation (GAG/DNA, IHC:Safranin-O/Alizarin-red, Cx43/aggrecan/Col I/II) was characterized.

GelAGE-HepSH microspheres(Ø0.5mm,Ø1mm) were placed in MEW scaffolds with spatial accuracy. Both hydrophilic and 5µm diameter fibre MEW scaffolds were unable to retain spheroids. Thicker MEW structures supported fusion into larger tissue constructs, with high expression of Cx43 cell signalling, cell migration and ECM deposition, seen to support both chondrogenic (68.2±4.3GAG/DNA) and osteogenic (8.6±0.7ALP/DNA) tissue formation. Spheroids in NaOH-treated scaffolds displayed limited secretion of sGAGs(11.9±2.1GAG/DNA), highlighting the importance of optimising both scaffold design and post-treatment to guide cellular development through cell-material interactions.

In conclusion, 3Dbioassembly of microspheres into MEW scaffolds provides a facile conversion of two biofabrication strategies to achieve high-throughput shape control to support tissue fusion and deposition of tissue specific ECM proteins.

Keywords
Melt electrowriting; Microfluidics; 3D-Bioassembly

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Abstract **#703**

A 3D-BIOASSEMBLY PLATFORM FOR PROBING SPHEROID TISSUE FUSION AND INTEGRATION

Gabriella Lindberg$_P$[1], Laura Veenendaal[1], Xiaolin Cui[1], Cesar Alcala[1], Mitch Durham[1], Gary Hooper[1], Khoon Lim[1], Tim Woodfield[1]

[1] *Christchurch Regenerative Medicine and Tissue Engineering Group, Uni. of Otago, New Zealand*

Corresponding author's email: Gabriella.Lindberg@otago.ac.nz

Cell spheroids represent a reproducible, high throughput strategy to generate miniaturised organs. However, the understanding of spheroid fusion and tissue integration remains a bit of an enigma. This study aimed to develop a 3Dbioassembly platform to study tissue-tissue interfaces in vitro. We systematically investigated the effect of cell source and biomaterial matrix components on biological functions including cell migration, signalling, fusion kinetics, tissue-quality and tissue-host integration.

Human mesenchymal stromal cells and articular chondrocytes were fabricated into Ø1mm spheroids using either biomaterial-free centrifugation[1,2] or 3Dbiofabrication of naturally derived hydrogels (vitreous humor;VH[3], allylated gelatin;GelAGE[4], and thiolated heparin;HepSH[5]). Thermoplastic cages(1x2x1mm) were 3Dprinted to hold two tissue spheroids adjacently (3w,TGF-β1). Cellular health (live/dead®/alamarBlue®) and tissue formation (GAG,DNA, IHC:Safranin-O,Cx43/Col I/II, PCR:Col I/II,Agg,Sox9) was characterized.

Coordinated migration was essential for MSC tissue fusion while HAC tissue-tissue interfaces were lacking both cells and collagen type II. Multicellular spheroids remodelled early fibro-cartilage to articular matrix. However, fusion between mature MSC and HAC tissues did not upregulate the chondrogenic capacity of HACs – highlighting the importance of direct cell contact over soluble cell signals (HAC-MSC:5.8±2.0 GAG/DNA, Multicellular:15.5±1.7 GAG/DNA). VH based spheroids offered relevant biomimetic cues to integrate with mature cartilage, while GelAGE-HepSH was enzymatically degraded with limited tissue forming - stressing the importance of optimising both bioactivity and degradation profiles of biomaterials for successful integrative repair strategies.

Overall, a facile 3Dbioassembly platform was introduced to study cartilage-cartilage interfaces, offering a powerful route to study both multicellular fusion mechanisms in developmental stages of organoid growth and tissue-host integrative repair strategies.

Keywords

Biomimetic hydrogels; Tissue Fusion; Co-culture

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Abstract **#704**

PHOTOCROSSLINKED GELMA/HYALURONIC ACID HYDROGELS AS ADIPOSE TISSUE IN VITRO MODELS

Matteo Pitton[P][1], Christian Urzi'[1], Nicola Contessi Negrini[2], Silvia Fare'[1]

[1] *Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Milan, Italy*

[2] *Department of Bioengineering, Imperial College London, London, UK*

Corresponding author's email: silvia.fare@polimi.it

3D in vitro models of pathological adipose tissue (AT) are gaining tremendous interest for the possibility of fabricating in vitro AT equivalents useful for drug screening and new therapies development [1]. Nevertheless, the development of a scaffold able to healthy and/or pathological cell culture and mimic AT, to obtain a reliable in vitro model, is still challenging. Here, gelatine (Gel) and hyaluronic acid (HA) were investigated to realise an in vitro 3D model able to mimic AT extracellular matrix. Gel and HA were chemically modified via methacrylation to obtain GelMA and HAMA [2]. GelMA, HAMA and GelMA/HAMA hydrogels were obtained by photocrosslinking, exposing the polymeric solution to UV rays (Irgacure 2959) or to visible light (Ru/SPS as photoinitiator). The obtained samples were investigated by chemical, physical, mechanical and biological characterization.

H-NMR confirmed GelMA and HAMA methacrylation (DoF = 40.42% and 52.5%). The hydrogels showed good stability at 37°C up to 3 weeks and mechanical properties comparable to native AT. In vitro tests (3T3-L1 preadipocytes) revealed no cytotoxic effects of GelMA and HAMA obtained by UV or visible light exposure. Preadipocytes embedded in GelMA and GelMA/HAMA hydrogels were cultured up to 14 days. Alamar Blue and Live&Dead proved an increase in 3T3-L1 viability and metabolic activity for samples obtained by UV exposure or visible light exposure (0.1/1 [mM/mM] Ru/SPS concentration).

The results demonstrate the suitability of GelMA and HAMA hydrogels as 3D AT models and the potential of visible light photocrosslinking as a promising alternative to UV light exposure.

Keywords

hyaluronic acid; crosslinking; gelatin

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Abstract **#705**

CHARACTERIZATION OF BMP-2-IMMOBILIZED POROUS MICROPARTICLES FOR EFFECTIVE OSTEOGENIC DIFFERENTIATION

Min Ji Kim[P][1], June-Ho Byun[2], Jin Ho Lee[3], Se Heang Oh[1]

[1] *Department of Nanobiomedical Science, Dankook University, Cheonan 31116, Korea*

[2] *Department of Oral and Maxillofacial Surgery, Gyeongsang National University School of Medicine, Jinju 52727, Korea*

[3] *Department of Advanced Materials, Hannam University, Daejeon 34054, Korea*

Corresponding author's email: seheangoh@dankook.ac.kr

Sustained release of signaling molecules from delivery systems is a common strategy for ensuring their prolonged bioactivity and for minimizing safety issues. However, residual toxic reagents, the use of harsh organic solvents, and complex fabrication procedures in conventional delivery systems are considered as critical challenges toward clinical use. In this study, we developed microparticles with leaf-stacked structure throughout entire particle (ELSS) which can allow sustained release of the growth factor [i.e., bone morphogenetic protein-2 (BMP-2)] as well as enhance bone regeneration due to their unique morphology. The ELSS particles can be prepared by clinically feasible materials and procedures. The morphology of ELSS particles, BMP-2 release from ELSS particles; and proliferation and osteogenic differentiation of human periosteum-derived cells (hPDCs) on BMP-2-immobilized ELSS particles were investigated.

Acknowledgments

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Keywords

Polycaprolactone; Porous microparticle; BMP-2

Abstract **#706**

3D PRINTING OF DOUBLE-POROUS PLA SCAFFOLDS FOR BONE TISSUE ENGINEERING

Davide Vandoni$_P$[1], Davide Pellegatta[1], Nicola Contessi Negrini[2], Silvia Fare'[1]

[1] *Department of Chemistry, Materials and Chemical Engineering G. Natta, Politecnico di Milano, Milan, IT*

[2] *Department of Bioengineering, Imperial College London, London, UK*

Corresponding author's email: silvia.fare@polimi.it

Additive manufacturing allows fabricating scaffolds with a single porosity level, obtained by spacing the filaments during printing (fused deposition modelling, FDM), that only allows for cells colonization and ingrowth in the porous structures [1]. We developed a novel technology to print double-porous PLA scaffold for bone regeneration by combining FDM (i.e., 3D scaffolds porosity) and a chemical blowing agent (CBA), to obtain microporous 3D filaments [2].

PLA and CBA were extruded (TTCBA-degradation, to form microporosity on filaments. Scaffolds were characterized by morphology, pore size and distribution, in vitro degradation, CaP nucleation, compressive tests, in vitro cytotoxicity (L929) and cytocompatibility (MC3T3-E1) tests.

SEM showed a correlation between CBA content and microporosity. 1 and 3% CBA scaffolds exhibited small pores amount; 5% CBA scaffold showed the lowest pores number with the smallest diameter; 7.5% samples had the highest pores number; 10% samples had the largest diameter (10-90µm). Compressive elastic modulus decreased increasing the microporosity. Degradation tests showed a significant weight loss for 10% CBA scaffolds, due to the higher pores content. The 1.5SBF treatment showed the scaffold ability to promote CaP nucleation. In vitro biological test showed high viability for all scaffolds.

FDM and CBA were coupled to fabricate double-porous PLA scaffolds. The 7.5% CBA content was found optimal and it allowed obtaining promising results, without compromise the mechanical properties and degradation kinetics.

Keywords

microporosity; biomimetic

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Abstract **#707**

CRYOPRESERVATION OF TESTICULAR TISSUE AND CELLS IN HUMAN AND ANIMAL SPECIES AND CHECK THE QUALITY OF SAMPLES AFTER RECOVERY

Sepideh Ashouri Movassagh[P1], Sanaz Ashouri Movassagh[2], Maryam Farghadan[1], Masoumeh Asadi[1], Meysam Ganjibakhsh[3], Samane Mahmoud Aghdam[1], Hedieh Rahmati[1]

[1] Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran.

[2] Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

[3] Royan Institute Department of Stem Cells and Developmental Biology, ACECR, Tehran, Iran.

Corresponding author's email: ashouri_sepideh@yahoo.com

AbstractBackground and Aim: Cryopreservation of tissues and germ cells is a way to maintain the fertility and reproduction of valuable and endangered species the aim of this study was to cryopreserve the testicular tissue and cells of different species while maintaining the quality of these samples. Materials and Methods: In this experimental study, Samples of human testicular tissue and various animal species (Mouse, Rat, Bovine and Sheep) were prepared and stored in small pieces or after enzymatic digestion and extraction of cells by slow freezing method. The quality of frozen tissues and cells after post freeze was assessed by their viability and culture.Results: The results showed that after thawing, an acceptable percentage of the cells were alive and cultured. Also, the study of gene expression in different stages of spermatogenesis by Real-time PCR method in human sample showed that the sex cells are well preserved in different stages of maturation.Conclusion: In this study, the quality and viability of testicular tissue and cells after thawing, was well preserved by the used method. The results of this research could be a way to preserve fertility in human and a way to preserve and reproduce valuable species.

Keywords

Cell; Testis; Cryopreservation

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Abstract **#708**

MICRO CONFINEMENT INDUCES STELLATE CELL ASSEMBLY, ACTIVITY, AND CORRALLING OF PANCREATIC CANCER CELLS

Stephanie Nemec$_P$[1], Kristopher Kilian[2]

[1] *School of Materials Science & Engineering, University of New South Wales Sydney, Australia*

[2] *School of Chemistry, Materials Science & Engineering, University of New South Wales Sydney, Australia*

Corresponding author's email: s.nemec@student.unsw.edu.au

Micro confinement cues can direct cellular interaction. Combining cancerous and non-cancerous in co-cultures contributes to cellular motility, localization, and organization. However, it is unclear how this milieu of signals influences the assembly of co-cultures on confined geometries. Pancreatic cancer is enriched with a cancer-associated fibroblast or stellate cellular population that when activated coordinates the deposition of extracellular matrix and can comprise over 90% of patient tumours. To observe the activity and coordination of stellate cells with pancreatic cancer, we confined an equal ratio co-culture population to protein-micropatterned hydrogels. We find that interfacial cues of the pattern edge influence stellate migration and corralling of cancer cells, especially in circle shapes. Surprisingly, enhancing convex curvature further activates and coordinates stellates yet the cells are free to migrate throughout the micropatterned region. Using computational modelling, we show the cellular localization is curvature-stress dependent for both cancer and stellate co-cultures. We also find that inhibiting mechanotransduction pathways decreases stellate activation and associated corralling. Our model also indicates interfacial cues influence collagen I production a key component of the stromal/desmoplasia reaction that decreases chemotherapeutic response in diseases such as pancreatic, breast, and liver cancers.

Abstract **#709**

PORCINE DERMAL MATRIX FOR SKIN TISSUE ENGINEERING: AN EXPERIMENTAL STUDY

Alexander Sotnichenko$_P$[1], Karina Melkonyan[1], Irina Gilevich[1,2], Sergey Bogdanov[1,2], Alexander Verevkin[1], Tatyana Rusinova[1], Yana Kozmay[1], Ilya Bykov[1], Denis Yshmarov[1], Sergey Gumenyuk[1], Vladimir Porhanov[1,2], Andrey Redko[1], Sergey Alekseenko[1]

[1] Kuban State Medical University, Krasnodar, Russia.

[2] Research Institute – Krasnodar Regional Hospital No. 1 named after Prof. S.V. Ochapovsky, Krasnodar, Russia

Corresponding author's email: alex24.88@mail.ru

Aim of the study was to develop optimal wound covering based on tissue-engineering technology using decellularized porcine skin matrix. Two decellularization protocols were examined: protocol No.1 based on the using of Triton X-100 and sodium deoxycholate, protocol No.2 based on sodium deoxycholate only. After characterization by histology, DNA quantification and in vitro cytotoxicity tests the protocol No.1 was chosen for further experiments. In vivo study was carried on 1 pig of the Landrace breed. A deep skin wound was created on the back by a dermatome. For closure the decellualrized and recellularized by dermal fibroblast matrices were used, and samples from wound were assessed histologically by retrieving those during early and long-term postoperative period.

Both matrices, decellularized and recellulatized, showed the tissue biocompatibility, the development of weak inflammatory reaction and the absence of a connective tissue capsule. The decellularized matrix was detached by day 12 revealed the connective tissue covering the wound. The recellularized scaffold underwent progressive in vivo remodeling resulted to form a full-layer skin. The main feature was the existence of healthy skin with growth of hair and developed skin appendages observed during the long-term period.

Finally, we present the approach of wound covering, based on decellularized dermal matrix, which was effectively recellulatized by fibroblasts, and subsequently promoted the formation of full-thickness skin that did not require additional coating.

Acknowledgements:The work was performed as part of a complex research (№AAAA-A16-116042550089-5 from 25.04.2016)

Keywords

tissue engineering; decellularization; biological scaffolds

Abstract **#711**

STRUCTURALLY-DISCOVERED TRANSCRIPTION FACTOR VARIANTS ACCELERATE AND STABILIZE REPROGRAMMING TO PLURIPOTENCY

Evgeniia Borisova[P][1,2], Ken Nishimura[2], Yuri An[1], Miho Takami[1,2], Jingyue Li[1,2], Dan Song[1], Mami Matsuo-Takasaki[1], Dorian Luijkx[1], Shiho Aizawa[2], Akihiro Kuno[3], Eiji Sugihara[4], Fumiaki Yumoto[5], Tohru Terada[6], Koji Hisatake[2], Yohei Hayashi[1]

[1] IPS cell advanced characterization and development team, RIKEN BioResource Research Center, Tsukuba, Japan

[2] Laboratory of Gene Regulation, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

[3] Laboratory of Animal Resource Center, Department of Anatomy and Embryology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

[4] Research and Development Center for Precision Medicine, Innovation Medical Research Institute, University of Tsukuba, Tsukuba, Japan

[5] The Institute of Materials Structure Science, High Energy Accelerator Research Organization in Tsukuba, Tsukuba, Japan

[6] Interfaculty Initiative in Information Studies, The University of Tokyo, Tokyo, Japan

Corresponding author's email: evgeniia.borisova@riken.jp

Somatic cells can be inefficiently and stochastically reprogrammed to pluripotent cells by exogenous expression of reprogramming factors, which are mostly transcription factors. Transcription factors cooperatively bind closed parts of the DNA to regulate downstream genes and to change epigenetic status toward reprogrammed cell states. However, current stagnation of reprogramming technology accounts for such drawbacks as a poor and slow yield of high-quality reprogrammed cells from primary somatic cells, which results in delays of medical applications. Natural transcription factors have bee used as reprogramming factors, but the effective functional alteration of reprogramming factors remains unexploited overall. Low competence of natural reprogramming factors might prevent the majority of cells to successfully and synchronously reprogram. Among reprogramming factors, Kruppel-like factor 4 (KLF4) plays central roles in reprogramming toward pluripotent state and other cell types.

Here we screened amino acid residues in the zinc-finger domain of KLF4 for enhanced reprogramming efficiency. Identified KLF4 mutant accelerated and stabilized reprogramming to pluripotency in both mouse and human somatic cells. Further, from all the amino acid residue variants of defined position, smaller amino acid residues in that position showed enhanced reprogramming activity. ChIP-seq and RNA-seq experiments revealed that the identified mutant bound to promoters of several pluripotency genes more and drove the gene expression of these genes during reprogramming. Molecular dynamics simulations predicted that this mutant formed additional interactions with DNA. Our study demonstrates that modifying amino acid residues of DNA-binding domain enables next-generation reprogramming technology with engineered reprogramming factors.

Keywords

Regenerative medicine; Bioengineered reprogramming factors; Reprogramming biotechnology

Abstract **#712**

A BIOINSPIRED BOTTLEBRUSH POLYMER FOR ARTICULAR CARTILAGE REGENERATION

Ivo Beeren$_P$[1], Lorenzo Moroni[1], Matt Baker[1]

[1] *Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands*

Corresponding author's email: i.beeren@maastrichtuniversity.nl

Articular cartilage is a hydrogel-like tissue, which enables humans to resist high repetitive loading cycles painlessly. The characteristic resilience arises from the bottle brushed glycosaminoglycan (GAG) organization and extruding of water by the tissue, which increases the strength during compression. Afterwards, the organized negatively charged GAGs rehydrate the tissue again for joint lubrication. During aging articular cartilage often loses both its GAG content and its ability to remodel. Therefore, the tissue's resistance to repetitive loading is diminished making it more susceptible to damage and osteoarthritis. Tissue engineers could recapitulate the lost biomechanical properties in newly developed hydrogels. However, hydrogels often suffer from low mechanical properties. Smartly designed bottle brushed polymers have interesting water uptake and mechanical properties for cartilage applications such as lubrication1 or strain-stiffening effects2. Therefore, we hypothesized that for cartilage applications a bioinspired bottle brushed polymer could offer a solution to resist cyclic loading and simultaneously maintain its lubrication function. We have designed a biohybrid polymer based on a hyaluronic acid backbone to which short RAFT polymerized side arms were coupled. Initially, we attached hydroscopic side arms to the backbone. To improve modularity of the hydrogel platform, we then switched towards designing activated side arms, which allows for facile customization of the platform for attaching cross-linkers or peptides. The bottlebrush polymers were characterized chemically, mechanically and physically. Furthermore, we tested injectability and cell viability of chondrocytes. Our results show that the design of the bottle brush and hydroscopicity have great impact on the mechanical properties and cell viability.

Keywords

bottle brush; biomimicry; injectability

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Abstract **#713**

THE DEVELOPMENT AND APPLICATIONS OF MILK DERIVED PROTEIN BASED SCAFFOLD FOR BONE REGENERATION

Hee Seok Yang$_P$[1]

[1] *Dankook University*

Corresponding author's email: hsyang@dankook.ac.kr

Recently, a number of studies have reported that bioactive peptides of food proteins induced positive biological activities. One of these candidates, casein is a well-characterized protein representing 80% of total milk proteins. The β-casein possesses various multifunctional biopeptides such as casein phosphopeptide, β-casochemotide-1 that bind metal ions including calcium and magnesium with high affinity and induce macrophage chemotaxis which is crucial for bone homeostasis and repairing bone fracture by improving the differentiation of mesenchymal stem cells (MSCs). Thus, we hypothesized that the reparation of a casein-based scaffold could be enhanced bone regeneration by various biopeptides. We designed a scaffold that casein was cross-linked with biocompatible polyvinyl alcohol (PVA) via a simple freeze-thaw method (milk-derived protein, MDP). Also, the MDP coated with 3,4-dihydroxyphenylalanine (L-DOPA) (MDP-DOPA) which had been known as a mussel adhesive protein for immobilizing adhesive proteins and cytokines for recruited cells. The MDP and MDP-DOPA groups proved indirectly the contribution of macrophages migration that RAW 264.7 cells were highly migrated toward MDP-DOPA which were contained biopeptide. To demonstrate in vivo bone regeneration via biological interactions between recruited cells including macrophages and MSCs by biopeptides, we transplanted both MDP and MDP-DOPA on mouse calvarial defect model (ø=4 mm). We confirmed new bone formation using a live micro-CT and histological study, MDP-DOPA group showed faster mineral deposition and higher bone density compared to MDP group. For these results, we demonstrated the effect of multifunctional biopeptide in casein that had great potential as biomaterials via the synergetic effect of casein and L-DOPA.

Keywords

Tissue Engineering; Biomaterials; Milk derived protein

Abstract **#714**

DISSECTING THE EFFECTS OF PRECONDITIONING WITH INFLAMMATORY CYTOKINES AND HYPOXIA ON THE ANGIOGENIC POTENTIAL OF MESENCHYMAL STROMAL CELL (MSC)-DERIVED SOLUBLE PROTEINS AND EXTRACELLULAR VESICLES (EVS)

Roberta Tasso[P][1], Cansu Gorgun[1,2], Davide Ceresa[2], Raphaelle Lesage[3], Federico Villa[2], Daniele Reverberi[2], Carolina Balbi[4], Sara Santamaria[1], Katia Cortese[1], Paolo Malatesta[1], Liesbet Geris[3,5], Rodolfo Quarto[1,2]

[1] *University of Genova, Italy*

[2] *IRCCS Ospedale Policlinico San Martino, Genova, Italy*

[3] *KU Leuven, Belgium*

[4] *Cardiocentro Ticino Foundation, Lugano, Switzerland*

[5] *University of Liège, Belgium*

Corresponding author's email: roberta.tasso@unige.it

Mesenchymal stromal cells (MSCs) are characterized by a regulatory phenotype and respond promptly to the environmental signals modulating their secretory activity. An appropriate preconditioning may induce MSCs to release secretomes with an enhanced regenerative potential. Secretomes are composed by both soluble factors and extracellular vesicles (EVs), whose functions could be altered differently by the preconditioning approach. Here we demonstrate that the MSC secretome is strongly modulated by the simultaneous stimulation with hypoxia and pro-inflammatory cytokines, used to mimic the harsh environment present at the site of injury. We observed that the environmental variations strongly influenced the angiogenic potential of the different secretome fractions. Upon inflammation, the pro-angiogenic capacity of the soluble component of the MSC secretome was strongly inhibited, regardless of the oxygen level, while the EV-encapsulated component was not significantly affected by the inflammatory stimuli. These effects were accompanied by the modulation of the secreted proteins. On one hand, inflammation-activated MSCs release proteins mainly involved in the interaction with innate immune cells and in tissue remodeling/repair; on the other hand, when MSCs are not exposed to an inflamed environment, they respond to the different oxygen levels modulating the expression of proteins involved in the angiogenic process. The cargo content (in terms of miRNAs) of the corresponding EV fractions was less sensitive to the influence of the external stimuli. Our findings suggest that the therapeutic efficacy of MSC-based therapies could be enhanced by selecting the appropriate preconditioning approach and carefully discriminating its effects on the different secretome components.

Abstract **#715**

NANOPLATFORMS DELIVERING MSC SECRETOME FOR SKIN WOUND HEALING

Barbara Blanco-Fernandez[P][1], Cristina Alcoholado[2], Antonio Villatoro[2], Cristina Antúnez-Rodríguez[3], Rick Visse[2], José Becerra[2], Elisabeth Engel[4,5,1], Soledad Perez-Amodio[1,4,5]

[1] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain

[2] Department of Cell Biology, Genetics and Physiology, University of Malaga, IBIMA, BIONAND, Malaga, Spain.

[3] Expansion and cell therapy unit, Center for Tissue and Cell Transfusion of Málaga (CTTC), Spain

[4] Materials Science and Metallurgical Engineering, Polytechnical University of Catalonia (UPC), Barcelona, Spain.

[5] CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain.

Corresponding author's email: bblanco@ibecbarcelona.eu

INTRODUCTION: Mesenchymal stem cells (MSCs) regulate cell processes by secreting growth factors, cytokines, chemokines, hormones and extracellular vesicles, known as secretome. This secretome has successfully exhibited therapeutic properties.1 Although secretome can be directly administered in the target site, its fast clearance is still challenging. Therefore, new efforts have been made to develop delivery platforms,1 that sustain secretome release and increase its retention time.

The aim of this work is to encapsulate secretome into nanoparticles (NPs) for achieving a sustained release and to evaluate their potential in wound healing.

EXPERIMENTAL METHODS: Secretome was obtained by culturing human umbilical cord MSCs under hypoxic conditions. Protein content was determined using a protein array. Secretome was encapsulated in poly(lactic-co-glycolic) (PLGA) NPs by double emulsification. NPs size and zeta potential were measured using a Zetasizer. The loading content and release was evaluated using a microBCA. The secretome integrity was also assessed. NPs cellular cytocompatibility was studied using human dermal fibroblasts and keratinocytes.

RESULTS: The most expressed bioactive molecules detected in the secretome were TIMP-2, TIMP-1, IL-6, IL-8, RANTES. NPs encapsulate between 7.2-13.5 μg/mg NPs, having sizes of 300-400 nm and zeta potentials below -20 mV. NPs were biocompatible and sustained the release for 7 days.

DISCUSSION AND CONCLUSIONS: The secretome composition evidenced its potential in wound healing. Secretome was successfully encapsulated in the NPs, showing a sustained release. Our results indicate that these nanoplatforms are promising wound healing agents.

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Keywords

nanoparticle; secretome; MSC

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Abstract **#716**

PHOTOCURABLE BIOPOLYMERS AS TUNABLE MATERIALS FOR PERSONALIZED SCAFFOLD ARCHITECTURE THROUGH ADDITIVE MANUFACTURING TECHNIQUES

Irene Carmagnola[1,2], Chiara Tonda-Turo[1,2], Annalisa Chiappone[3,2], Giuseppe Melilli[4], Gianluca Ciardelli[1,2,5], Marco Sangermano[3,2], Minna Hakkarainen[6]

[1] *Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy*

[2] *PolitoBIOMed Lab, Politecnico di Torino, Torino, Italy*

[3] *Department of Applied Science and Technology, Politecnico di Torino Torino, Italy*

[4] *Institute of Chemistry of Nice, Université Côte d'Azur, France*

[5] *Department for Materials and Devices of the National Research Council, Institute for the Chemical and Physical Processes (CNR-IPCF UOS), Pisa, Italy*

[6] *KTH Royal Institute of Technology, Department of Fibre and Polymer Technology, Stockholm, Sweden*

Corresponding author's email: irene.carmagnola@polito.it

Recent progresses in Tissue Engineering are directed towards the development of technologies able to provide patient specific scaffolds. Additive manufacturing (AM) techniques combining with suitable materials are able to create a physiological milieu for cell growth. As the development of bio-based biocompatible and sustainable photocurable materials for 3D printing is a stringent request to employ these techniques in biomedical applications, methacrylate biopolymers has been synthetized and exploited as photocurable materials for different AM approaches [1][2]. Commercial chitosan (CS) and carboxymethyl cellulose (CMC) were chemically modified using methacrylic anhydride reagent. Methacrylated CS was then mixed with β-glycerol phosphate salt to impart a thermally-induced phase transition and produce a chitosan-based hydrogel as bioink for cell encapsulation and 3D bioprinting of well-defined scaffold architectures. In vitro tests performed using different cell lines (NIH/3T3, Saos-2, SH-SY5Y) confirmed the absence of cytotoxic degradation products and the excellent biocompatibility of the developed hydrogel. Cellularized 3D structures were then obtained through 3D bioprinting technology confirming the hydrogels processability and its unique biological properties.

Aqueous formulations based on methacrylate CMC were easily 3D-printed through a Digital Light Processing (DLP) apparatus to produce 3D shaped hydrogels with excellent swelling ability and mechanical properties. Envisaging the application of the hydrogels in the biomedical field, cytotoxicity is also evaluated. The light-induced printing of cellulose-based hydrogels represents a significant step forward in the production of new DLP inks suitable for biomedical applications.

Here, a platform of photocurable biopolymers were then proposed as promising materials to recreate complex living tissues through AM techniques.

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Abstract **#717**

UNDERSTANDING THE MECHANISMS OF IN SITU HEART VALVE TISSUE ENGINEERING AFTER BIORESORBABLE PULMONARY VALVE IMPLANTATION IN SHEEP

Bente de Kort[P][1,2], Arturo Lichauco[1], Julia Marzi[3,4,5], Eva Brauchle[3,4,5], Sylvia Dekker[1], Aurelie Serrero[6], Martijn Cox[6], Katja Schenke-Layland[3,4,5], Carlijn Bouten[1,2], Anthal Smits[1,2]

[1] Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

[2] Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands

[3] Department of Women's Health, Research Institute for Women's Health, Eberhard Karls University Tübingen, Tübingen, Germany

[4] Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University Tübingen, Germany

[5] NMI Natural and Medical Sciences Institute, University of Tübingen, Reutlingen, Germany

[6] Xeltis B.V., Eindhoven, The Netherlands

Corresponding author's email: b.j.d.kort@tue.nl

In situ heart valve tissue engineering utilizes the regenerative response of the human body to create a living, functional replacement valve from a resorbable, synthetic and off-the-shelf available graft. Recently, preclinical studies using electrospun supramolecular elastomeric valvular grafts have shown that this principle, also known as Endogenous Tissue Restoration (ETR), enables in situ regeneration of pulmonary valves with sustained long-term functionality in vivo1,2. However, detailed knowledge on the underlying mechanisms of the material-driven endogenous regenerative response remains elusive. Therefore, the goal of the present study was to gain a mechanistic understanding of these processes by combining immunohistochemistry, using a comprehensive sheep-specific antibody panel3, and Raman spectroscopy for spatiotemporal analysis of in situ tissue-engineered heart valves with follow-up up to 24 months. Detailed knowledge on graft resorption, neo-tissue formation, as well as the involved cellular influences was obtained. Spectral changes of graft material 2 months post-implantation, indicated starting degradation in the arterial conduit and base region of the leaflet, correlating with the presence of foreign body giant cells at these iNOS-rich, tissue remodeling sites. Interestingly, in the base region giant cells were located within the graft whereas in the conduit material giant cells remained on the outer layers with only limited infiltration into the scaffold material. Extracellular matrix components, such as collagens and elastin were formed in a highly heterogeneous fashion. Taken together, these findings enhance the understanding of material-driven regeneration and highlight the importance of the micro-environment and hemodynamic loading on the cellular response towards a biomaterial.

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Abstract **#718**

BIODEGRADABLE SCAFFOLD FOR MULLERIAN ANOMALY: FABRICATION AND IN-VITRO STUDY TO IMPROVE WOMEN'S REPRODUCTIVE HEALTH

Atul Kumar Ojha[P][1], Ragavi Rajasekaran[1], Abir Dutta[1], Sunita Sharma[2], Kamal Oswal[3], Santanu Dhara[1], Koel Chaudhury[1]

[1] *School Of Medical Sciences and Technology, Indian Institute of Technology I.I.T. Kharagpur, India*

[2] *Institute of Reproductive Medicine, Salt Lake, Kolkata, India*

[3] *Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, Kolkata, India*

Corresponding author's email: ojha.kratul@gmail.com

Mullerian Anomalies (MA) comprise all congenital disorders that affect the development of the Mullerian duct into the female reproductive tract in the fetus. It manifests itself as defect in organogenesis, vertical or lateral fusion and septal resorption causing this congenital uterine malformation. Tissue engineering for female reproductive organs using various biomaterials might help to overcome this complex anomaly. The study aims to engineer a bilayer scaffold using combinatorial approach of electrospinning on a printed structure utilizing silk fibroin (SF) and Polycaprolactone (PCL) in homogenous blend form. Briefly, different polymer ratio was chosen and optimized to fabricate scaffold by direct printing followed by electrospinning of nanofibrous layer. The electrospun substrate presented nanofibrous architecture ranging from 150 nm to 600 nm similar to native extracellular matrix (ECM) structure. 3D printed substrate supported the structure in terms of size/ shape retention that eventually facilitated enhancement in mechanical properties of the scaffold. SEM microscopy images showed nano and microstructural analysis of scaffold as well as pore distribution. Physico-chemical characterization using XRD, FTIR, and Raman spectroscopy confirmed the presence of SF and PCL. Lysozyme degradation assay showed 16% degradation after 30 days (with specific composition of SF/PCL x/y). Contact angle measurement evidenced hydrophilicity with contact angle value of 40 degree supporting 80% water absorption in less than 10 hrs. In vitro studies using fibroblast cells demonstrated enhanced cell viability, cell anchoring, migration and proliferation. It is envisioned that the developed scaffold would be beneficial to improve women reproductive health, especially those who suffer from MA

Abstract **#719**

CUSTOM-MADE THERMO-SENSITIVE HYDROGELS AS VEHICLES TO LOCALLY DELIVER IBUPROFEN-LOADED MESOPOROUS CARBONS FOR A SUSTAINED AND PROLONGED PAYLOAD RELEASE.

Monica Boffitop[1], Rossella Laurano[1], Dimitra Giasafaki[2], Theodore Steriotis[2], Athanasios Papadopoulos[2], Chiara Tonda-Turo[1], Georgia Charalambopoulou[2], Gianluca Ciardelli[1]

[1] *Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Turin, Italy*

[2] *National Center for Scientific Research "Demokritos", Athens, Greece*

Corresponding author's email: monica.boffito@polito.it

Ordered mesoporous carbons (OMCs) have been proven to be excellent drug carriers, thanks to their high loading capacity, good cytocompatibility and easy surface functionalization. However, OMCs need to be appropriately incorporated in dosage forms to allow their retention in target tissues for the requested time to completely release their cargo. To overcome this drawback, we embedded ibuprofen (IBU)-loaded spherical and rod-shape OMCs (approx. 120 and 500 nm, respectively, 100% IBU loading) into thermo-sensitive hydrogels (15-20% w/v, up to 10 mg/ml OMC concentration) based on a custom-made amphiphilic poly(ether urethane) (PEU) containing Poloxamer®407 blocks (Mn 72kDa)[1]. OMC incorporation did not worsen the overall gelation potential of PEU aqueous solutions, which still exhibited fast sol-to-gel transition under physiological conditions (few minutes). Rheological characterization highlighted that OMC geometrical features and surface coating with sodium dodecyl sulfate (SDS, 1% w/v to improve OMC dispersibility in aqueous media) played a key role in influencing the progressive organization of the polymeric chains into a gel network. Indeed, spherical OMCs better integrated into the gel network, whereas SDS heads took part to the gelation process through the formation of hydrogen bonds with PEU chains. The developed hybrid formulations released IBU at a slower rate compared to gels loaded with IBU as such (85% vs. 100% IBU released within 3 weeks), thus effectively working as injectable depots progressively releasing their cargo in loco and improving OMC residence-time in the target tissue. Hence, the engineered platform could effectively solve issues related to multiple dosing providing a long-lasting therapeutic device.

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Abstract **#720**

BIOSURFACTANT-BASED COATINGS ON MEDICAL-GRADE SILICONE FOR BIOFILM FORMATION INHIBITION

Irene Carmagnola[P][1,2], Chiara Ceresa[3], Letizia Fracchia[3], Gianluca Ciardelli[1,2,4], Valeria Chiono[1,2]

[1] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy

[2] POLITO BIOMedLAB, Politecnico di Torino, Turin, Italy

[3] Department of Pharmaceutical Sciences, Università del Piemonte Orientale "A. Avogadro", Novara, Italy

[4] Department for Materials and Devices of the National Research Council, Institute for the Chemical and Physical Processes (CNR-IPCF UOS), Pisa, Italy

Corresponding author's email: irene.carmagnola@polito.it

Given their effectiveness in improving patient health and quality of life, the use of silicone-based medical devices significantly increased over the years. Unfortunately, they can also act as a substrate for microbial colonization and biofilm development. Biosurfactants have recently emerged as a new generation of anti-adhesive and anti-biofilm agents [1][2]. In this work, an approach via plasma technology was proposed to covalent graft BSs on medical grade silicone substrates. Argon plasma treated silicone samples were aminolized using 3-aminopropyltriethoxysilane (APTES) to provide amino-functional groups exploited to covalently graft BSs via carbodiimide chemistry. Various characterization techniques (contact angle analysis, XPS and colorimetric assay) were used to confirm the success of each functionalization step.
The anti-adhesive/anti-biofilm activities of BS covalent grafting on modified silicone were tested against Candida albicans and Staphylococcus aureus strains. The evaluations were carried out after 1.5h (fungal adhesion phase) and 24h, 48h, 72h (intermediate and mature phases of fungal and bacterial biofilm formation). C. albicans adhesion was inhibited of 86% whereas biofilm formation was reduced in term of biomass and metabolic activity of 80% and 78% respectively, at the last time-point (72h). Biomass and metabolic activity of S. aureus biofilms were respectively reduced of 98% and 93% at 72h.The effect of these modified surfaces resulted to be constant, or even incremental, over time. In conclusion, a strategy to covalently graft BS on silicone substrates has been proposed and the anti-adhesive and anti-biofilm activity of the coating was demonstrated.

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Abstract **#722**

DEVELOPMENT OF NOVEL HUMAN EPITHELIAL MUCOSAL TISSUE MODELS AND THE ROLE OF TISSUE SPECIFIC FIBROBLASTS

Steven Bradbury[P][1], Amy Harding[2], Lydia Costello[1], Matthew Freer[1], Nicola Fullard[1], Helen Colley[2], Stefan Przyborski[1,3]

[1] *Durham University*

[2] *The University of Sheffield*

[3] *Reprocell Europe*

Corresponding author's email: s.m.j.bradbury@durham.ac.uk

Introduction

Epithelial tissues such as skin, intestine and the oral mucosa share a common, highly conserved structure however, amongst different tissue types the surface epithelium differs and is highly specialised according to its function.

We hypothesise that signalling from the fibroblasts within the submucosal compartment is critical to the differentiation of the overlying epithelial layer. Using advanced 3D tissue culture and tissue engineering technologies we propose to investigate whether these signals are tissue-specific and assess the paracrine and juxtacrine influences between these cell populations.

Methods

Reconstructed human full-thickness skin, intestinal and oral mucosal models have been developed utilising both primary cells and existing cell lines; fibroblasts are seeded in Alvetex® Scaffold inserts forming a robust submucosa on top of which epithelial cells are seeded, which subsequently differentiate.

Results

Our tissue models demonstrate structural morphology resembling the structure of native skin, intestine and buccal mucosa. Fibroblasts produce endogenous extracellular matrix proteins which provides support for the overlying epithelia. Extensive characterisation of the models shows evidence of basement membranes and multiple other structural and functional features shared by the native tissue. When epithelial cells were cultured with non-tissue matched fibroblasts this had a significant influence on the differentiation and morphology of the overlying epithelia. Intestinal epithelial cells showed greater polarisation. Oral epithelial cells underwent a more epidermal-like differentiation pathway producing a terminally differentiated stratum corneum.

Conclusion

We provide preliminary evidence that tissue-specific fibroblasts are required for the formation of fully-differentiated mucosal tissue constructs and alternative fibroblasts sources can alter epithelial cell differentiation.

Keywords

Differentiation; Enabling technologies; In vitro tissue models

Abstract **#724**

BONE CEMENTS FOR LOCAL GENTAMICIN DELIVERY

Eliza Tracuma[P][1], Dagnija Loca[2,1], Marite Skrinda[1], Janis Locs[2,1]

[1] Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Pulka 3, Riga, LV-1007, Latvia

[2] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

Corresponding author's email: eliza.tracuma@rtu.lv

INTRODUCTION: The alpha phase of tricalcium phosphate (α-TCP) is often used as a main constituent of calcium phosphate bone cements (CPC) [1]. One of the main advantages of CPC is their application through minimally invasive surgical procedures. Even through, post-surgery infection risks are possible, usually precluded through systemic antibiotic therapy [2]. Gentamicin sulphate (GENTA) is commonly antibiotic of choice due to its wide antibacterial spectrum [1]. The aim of the current study was to prepare controlled release GENTA delivery systems based on the CPC. For this purpose, GENTA was microencapsulated in poly-L-lactic acid (PLA) matrix and prepared vehicles were dispersed within the CPC matrix.
METHODS: Microcapsules were prepared by water-in-oil-in water (W1/O/W2) double emulsion technique. CPC composites were prepared through mixing α-TCP powder and GENTA/PLA microcapsules with sodium containing salt solutions.
RESULTS: Analysis of the prepared microcapsules showed that upon encapsulation of GENTA in the PLA matrix it is possible to obtain microcapsules in which the total GENTA content equals up to 3.27±0.2 wt.%. Drug release profiles from CPC/microcapsule composites indicated that GENTA has substantial affinity to the CPC matrix, influencing the active substance release in the water medium.
DISCUSSION & CONCLUSIONS: During the research it was established that only part of the drug could be extracted from the CPC, suggesting that sulphate ions of GENTA most likely bind to the cement via van der Waals interactions.
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Keywords

Drug Delivery; Calcium Phosphate Bone Cements; Biomaterials

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Abstract **#725**

ECM-MIMETIC 3D HYDROGEL MODELS FOR PROBING CELL AGGREGATION INFLUENCE ON DRUG SCREENING

Maria Monteiro$_P$[1], Vítor Gaspar[1], João Mano[1]

[1] *Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal*

Corresponding author's email: m.monteiro@ua.pt

3D in vitro tumor models bioengineering in ECM-mimetic hydrogels that recapitulate the tumor microenvironment provide a unique opportunity for accelerating therapies preclinical screening [1,2]. During ECM-biomaterials design stages, the selection of cell-laden hydrogel or hydrogel-3D tumor spheroid platforms remains however to be fully elucidated. Investigating the effect of differential cellular arrangement within hydrogels is paramount for robust anti-cancer therapeutics evaluation is such platforms [3]. To evaluate this, herein we bioengineered 3D spheroid microtumors or cell-laden osteosarcoma models in viscoelastic GelMA or Matrigel ECM-mimetic hydrogels, and evaluated cancer cells/microtissues maturation, as well as their response to an ALK targeted chemotherapeutic.

3D microtumors laden in ECM-mimicking hydrogels exhibited spheroidal morphology, exhibited the characteristic necrotic core found in vivo and presented significant sprouting in all ECM-mimetic hydrogels tested, thus mimicking metastases occurring at advanced disease stages. Cell-laden hydrogels did not exhibit cellular sprouting nor the formation of dense solid microtumors with necrotic core. Additionally, cell-laden hydrogels exhibited higher susceptibility to Lorlatinib treatment than 3D spheroid-laden hydrogels models. Hence, during in vitro maturation, osteosarcoma spheroid-laden hydrogels better recapitulated key features of native tumors and exhibited increased resistance to chemotherapeutics. These responses demonstrate that hydrogel-laden 3D tumor spheroids may provide a more suitable 3D microenvironment for in vitro tumor modelling and drug screening than their cell laden counterparts. Such findings highlight the importance of carefully evaluating different cellular aggregation states and microenvironments when designing ECM-mimetic hydrogels for in vitro disease modeling and for screening candidate anti-cancer therapeutics so as to extrapolate more realistic performance data.

Keywords

Hydrogels; 3D Spheroids; Drug-screening

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Abstract **#726**

REGENERATIVE THERAPY OF BRONCHIAL FISTULA

Irina Gilevich[P][1,2], Alexander Sotnichenko[2], Elena Kolomiytceva[1], Karina Melkonyan[2], Aleksey Kovalenko[1], Irina Muntyan[1], Igor Polyakov[1], Vladimir Porhanov[1]

[1] *Scientific Research Institute - Krasnodar Regional Clinic Hospital #1, Krasnodar, Russia*

[2] *Kuban State Medical University, Krasnodar, Russia*

Corresponding author's email: giliv@list.ru

We attempted to develop a new treatment for bronchial fistula using regenerative therapy including autologous platelet lysate and allogeneic dermal fibroblasts.

METHODS:This study included 34 patients with bronchial fistula after lung surgery. The regenerative product, consisting of human allogenic dermal fibroblasts suspension and autologous platelet lysate, was injected into the cavity of the fistula through a bronchofibroscope every second day totally five times. Hystological examination of cells from biopsies was performed after each bronchoscopy.

Human allogeneic dermal fibroblast were obtained from GMP laboratory. The total number of cells used in the one injection was 2 million per ml.

Platelet rich plasma was obtained by two-step centrifugation the patient's blood and collecting platelet concentrate suspended with plasma of total volume 10 ml. Freeze/thaw cycles were used for activation platelet. The aliquots of 2 ml platelet lysate were stored at −80oC until use.

RESULTS: The bronchial fistula was localized in main bronchus in 23 (67%) cases, in peripheral bronchus – 8 cases. From 34 patients 29 had the diameter of fistula less 10 mm, and in 5 cases the full failure of bronchial stump was determined.

All patients underwent bronchoscopy after treatment. All fistulas closed within 1 months after beginning of regenerative therapy. Bronchoscopic image confirmed fistula healing. No recurrence developed after definitive treatment. There were no adverse events. The histological examination revealed the formation of granulation tissue, metaplastic squamous epithelium.

CONCLUSIONS:Based on the results of this study, a regenerative therapy of bronchial fistula appears to be a promising treatment modality.

Keywords

Bronchial fistula; fibroblasts

Abstract **#727**

ANGIOGENESIS IS DIFFERENTIALLY MODULATED BY PLATELET-DERIVED PRODUCTS

Sarah Berndt$_P$[1], Gilles Carpentier[2], Antoine Turzi[3], Frédéric Borlat[4,5], Muriel Cuendet[4,5], Ali Modarressi[6,7]

[1] *RegenLab, University of Geneva*

[2] *Gly-CRRET Research Unit 4397, Paris-Est Créteil University, France.*

[3] *RegenLab*

[4] *School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland.*

[5] *Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Geneva, Switzerland.*

[6] *Department of Plastic, Reconstructive and Aesthetic Surgery, Geneva University Hospitals, Geneva, Switzerland.*

[7] *Faculty of Medicine, Geneva University, Geneva, Switzerland.*

Corresponding author's email: sberndt@regenlab.com

Key angiogenesis steps imply fine tuning of endothelial cell activities: proliferation, differentiation and migration to settle new vessel growth. Physiologically, platelets and endothelium, through their proximity, interact to support angiogenesis. In tissue regeneration, platelet-derived products can trigger therapeutic angiogenesis through autologous growth factors, cytokines and chemokines delivery. In this study, we evaluated and compared the angiogenic modulation achieved by three different platelet-derived preparations: platelet-rich-plasma (PRP), PRP-hyaluronic acid (HA) or platelet lysates (PL) at various concentrations (5-40%).

We first evaluated their biological activities on HUVEC metabolism, viability, senescence and tube formation capacities on a 2D matrigel. PRP and PRP-HA exhibited comparable biological stimulation while PL showed the lowest activity. To mimic the complexity of sprouting angiogenesis, we used a 3D angiogenic model: the fibrin bead assay (FBA). PRP and PRP-HA stimulated all steps of the angiogenic process to promote a massive sprouting of a branched microvessel network (from 4.0 (control) to 13.6-fold stimulation of total microvessel length for PRP and from 2.2 (control) to 12.6-fold stimulation for PRP-HA. PL show a milder angiogenic response (from 2.2 (control) to 9.0-fold stimulation). PL did not allow proper microvessel formation but rather promoted a stimulation of cell proliferation. Secretome profiling revealed modulations of 26 human proteins (angiogenic cytokines and chemokines, pro and anti- angiogenic growth factors and their receptors, matrix metalloproteinase and serine protease receptor) upon platelet-derived products treatment.

These in vitro experiments present PRP and PRP-HA as safe and effective cell therapy systems when a sustained therapeutic angiogenesis is needed.

Abstract **#728**

THE BIOPACER- TISSUE ENGINEERED CARDIAC MICROFIBERS FOR THE TREATMENT OF HEART BLOCK

Stavroula Kyriakou$_P$[1,2], Hans Keijdener[1,2], Gunter Kerst[3], Petra Mela[4], Stefan Jockenhoevel[1,2]

[1] Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Forckenbeckstraße 55, 52074 Aachen, Germany

[2] Aachen-Maastricht Institute for Biobased Materials, Faculty of Science and Engineering, Maastricht University, Brightlands Chemelot Campus, 6167 RD, Geleen, The Netherlands

[3] Department of Pediatric Cardiology, University Hospital of RWTH, Aachen, Germany

[4] Department of Mechanical Engineering, Technical University of Munich, Boltzmannstr. 15, 85748 Garching, Germany

Corresponding author's email: kyriakou@ame.rwth-aachen.de

The atrioventricular heart block is a pathological situation that constitutes a significant burden in pediatric heart surgery. The aim of the project is the replacement of technical pacemaker systems with biological pacemaker systems made from the patient's own cells with the potential of growing along in the child's organism to reduce the repetitive operations. A mixture of human iPSC-derived cardiomyocytes and human umbilical vein endothelial cells are embedded in fibrin gel using a dual injection head system. Accordingly, fibroblasts are embedded in fibrin gel and the extruded fiber is coupled with the cardiomyocyte- endothelial cell fiber. Both fibers are twisted and cultivated for 14 days while conditioned in a cyclic strain bioreactor. During the cultivation the 2 fibers fused forming a mechanically stable construct. Connexin 43 staining showed the conductive capacity and electrical coupling of the fiber in the aligned cardiomyocytes. The endothelial cells formed capillary-like structures, as indicated by CD31 staining. Furthermore, the collagen deposition by fibroblasts mechanically strengthened the construct. Last but not least, the electrophysiological recordings suggested that the Biopacer construct could accomodate a transition up to 180 bpm which is very promising for the future ex vivo implantation and animal studies. To fulfill this aim, our future steps include the development of a multifunctional protective shield around the Biopacer which will mechanically support the construct during the implantation. In addition, the encapsulation of immunosuppressive and endothelialization promoting factors are about preventing the patient fibrotic response and contributing to the Biopacer connection to the blood circulation respectively.

Keywords

Atrioventricular block; Cardiomyocytes; Fibrin hydrogel

Abstract **#729**

SUITABLE SCAFFOLDS FOR DIFFERENTIATION OF RESPIRATORY EPITHELIAL CELLS ON BIOHYBRID IMPLANTS

Anja E. Luengen$_P$[1], Caroline Kniebs[1], Franziska Neukamp[2], Eva Miriam Buhl[3], Christian G. Cornelissen[4,1], Stefan Jockenhoevel[1,5], Anja Lena Thiebes[1]

[1] *Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany*

[2] *Department of Cardiovascular Engineering (CVE), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany*

[3] *Institute of Pathology, Electron Microscopy Facility, RWTH Aachen University Hospital, Aachen, Germany*

[4] *Clinic for Pneumology and Internal Intensive Care Medicine (Medical Clinic V), RWTH Aachen University Hospital, Aachen, Germany*

[5] *AMIBM-Maastricht University, Geleen, The Netherlands*

Corresponding author's email: luengen@ame.rwth-aachen.de

Patients diagnosed with unresectable airway stenosis often face limited treatment options. In previous studies, a biohybrid airway stent has been invented to treat stenoses caused by bronchotracheal cancer [1]. A nitinol stent is combined with a polymer cover to avoid tumor ingrowth. To maintain the mucociliary clearance throughout the luminal stent surface, the aim of this study was the exploration of different stent cover materials to promote differentiation of autologous respiratory epithelial cells inside the stent.

We examined differentiation of human respiratory epithelial cells (HREs) on different scaffold materials based on polytetrafluoroethylene, polycarbonate urethane (PCU), polyamide, polycarbonate, a collagen-elastin mixture (Cartimaix, Matricel) and a Cartimaix-PCU combination. PET-inserts were used as positive control. After one week of proliferation on different scaffolds in a liquid-liquid-interface, cell differentiation was stimulated in air-liquid-interface (ALI) for four weeks prior to analysis by electron microscopy, PAS reaction and immunohistochemistry. Furthermore, PC-9 tumor cell migration through Cartimaix-PCU was examined. We could prove mucociliary differentiation on Cartimaix, Cartimaix-PCU and controls whereas cells formed a confluent monolayer without ciliation on pure PCU. Other materials did not lead to formation of confluent cell layers. Tumor cells did not migrate through Cartimaix-PCU. In our study, we could show that Cartimaix provides suitable properties to be used as scaffold for epithelial cell differentiation. This evaluation of potential stent cover materials regarding their ability to encourage epithelial differentiation features a first step towards engineering biohybrid constructs for treatment of airway stenosis comprising ciliated epithelium that can support an improved mucociliary clearance in vivo.

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Abstract **#730**

SOUND-INDUCED MORPHOGENESIS FOR RAPID ORCHESTRATION OF MULTICELLULAR SYSTEMS

Tiziano Serra[P][1]

[1] *AO Research Institute Davos*

Corresponding author's email: tiziano.serra@aofoundation.org

Morphogenesis, a complex process, ubiquitous in developmental biology and tissue regeneration, is based on self-patterning of cells. Spatial patterns of cells, organoids, or inorganic particles can be forced on demand using acoustic standing waves, such as the Faraday waves. This technology allows tuning of parameters (sound frequency, amplitude, chamber shape) under contactless, fast and mild culture conditions, for morphologically relevant tissue generation. We call this method Sound Induced Morphogenesis (SIM). In this work, we use SIM to achieve tight control over patterning of endothelial cells and mesenchymal stem cells densities within a hydrogel, with the endpoint formation of vascular structures. Here, we first parameterize our system to produce enhanced cell density gradients. Second, we allow for vasculogenesis after SIM patterning control and compare our controlled technology against state-of-the-art microfluidic culture systems, the latter characteristic of pure self-organized patterning and uniform initial density. Our sound-induced cell density patterning and subsequent vasculogenesis requires less cells than the microfluidic chamber. We advocate for the use of SIM for rapid, mild, and reproducible morphogenesis induction and further explorations in the regenerative medicine and cell therapy fields.

Abstract **#731**

USING B-PEPTIDES TO CONTROL SELF-ASSEMBLING HYDROGEL STIFFNESS AND PORE SIZE WITHOUT CROSSLINKING

Meg McFetridge[P][1], Mark Del Borgo[1], Ketav Kulkarni[2], Sharon Ricardo[1], Mibel Aguilar[2]

[1] *Department of Pharmacology, The Monash Biomedicine Discovery Institute,Monash University*

[2] *Department of Biochemistry and Molecular Biology, The Monash Biomedicine Discovery Institute,Monash University*

Corresponding author's email: meg.mcfetridge@monash.edu

Self-assembling hydrogels hold potential to revolutionise the way we deliver therapeutics which are not orally available, as they can be injected directly to the desired location and provide their cargo protection from clearance. The porosity and stiffness of a hydrogel are critical to its function as a depot for therapeutic molecules, as they influence the release profile and potential location of injection, respectively. Control of porosity and stiffness are typically achieved through covalent crosslinking; however this can be damaging to delicate cargo and unsuitable for injection in situ.

According to previously described methods, N-acetylated lipidated tripeptide monomers were synthesised and purified[1, 2]. Peptide monomers of different compositions were mixed before hydrogel gelation, stiffness and injectability were measured with an Anton-Paar MCR 302 Rheometer.

We have developed a self-assembling hydrogel with controlled stiffness in the absence of crosslinking, by making subtle changes to the peptide composition. We hypothesise that pore size can be manipulated in a similar fashion, and we aim to characterise the relationship between peptide composition, stiffness and pore size. Preliminary results suggest that pore size and stiffness can be controlled independently in this system, which is unique amongst self-assembling hydrogels, Independent control of hydrogel porosity and stiffness without covalent crosslinking substantially broadens the scope of potential applications, such as tissue engineering, mechanobiology studies and controlled drug delivery.

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Abstract **#732**

FREEFORM PRINTING OF TUMOR INVASION MODELS WITHIN CELL-LADEN MICROGEL MATRICES

Thomas G Molley$_P$[1], Gagan K Jalandhra[1], Stephanie R Nemec[1], Aleczandria S Tiffany[2], Brendan A. C. Harley[2], Tzong-tyng Hung[1], Kristopher A. Kilian[1]

[1] *University of New South Wales*

[2] *University of Illinois Urbana-Champaign*

Corresponding author's email: t.molley@unsw.edu.au

The tissue microenvironment contains a complex assortment of multiple cell types, matrices, and vessel structures. Emulating this complex and often hierarchical organization in vitro has proved a considerable challenge. Recently, the method of bioprinting into granular materials has emerged with tremendous potential for tissue fabrication. Here, we demonstrate the first example of tumor microenvironments formed through direct writing of both vasculature channels and tumor cell aggregates, within a cell-laden microgel matrix. Photocrosslinkable microgels provide control over local and global mechanics, while enabling the integration of virtually any cell type. Using computational fluid dynamics, we show that removal of a sacrificial Pluronic ink defines vessel-mimetic channel architectures for endothelial cell linings. Pairing this vasculature with 3D printing of melanoma aggregates, we find that tumor cells migrated into the prototype vasculature as a function of spatial location, thereby providing a measure of invasive potential. The integration of perfusable channels with multiple spatially defined cell types provides new avenues for modelling development and disease, with scope for both fundamental research and drug development efforts.

Abstract **#733**

THE EVALUATION OF TISSUE BIOCOMPATIBILITY OF TISSUE-ENGINEERED TRACHEA IN NON-HUMAN PRIMATES

irina Gilevich$_P$[1,2], Alexander Sotnichenko[2], Karina Melkonyan[2], Igor Polyakov[1], Vladimir Porhanov[1,2], Vladimir Chekhonin[3]

[1] *Scientific Research Institute - Krasnodar Regional Clinic Hospital #1, Krasnodar, Russia*

[2] *Kuban State Medical University, Krasnodar, Russia*

[3] *N. I. Pirogov National Research Medical University, Moscow, Russia*

Corresponding author's email: giliv@list.ru

The aim of the study was to evaluate the biological compatibility of the tissue-engineered trachea based on a polyethylene terephthalate (PET) scaffold and allogenic mesenchymal stem cells (MSC) in experimental non-human primates.
METHODS:The study was carried out on 6 nonhuman adult male primate Papio hamadryas at Research Institute of Medical Primatology. The tissue-engineered trachea had composed of synthetic PET 3D-scaffold seeded with primate allogenic MSCs.
After 72 hours cultivation in the rotating bioreactor the samples of tissue-engineered trachea was used for subcutaneous heterotopic implantation into the scapular area of the back for local reaction. The samples existed from heterotopic position were removed after 30 days and histological examination was done.
RESULTS:The surgical intervention proper had no negative effect on animal status. Before the implantation cell viability on scaffolds was 73.4±10.2%. Morphological analysis of tissue-engineered samples showed chaotically located synthetic filaments to which cells adhered. The morphological evaluation of implanted samples showed a fine capsule, which enveloped the implanted scaffold; the capsule was presented by compact fibrous connective tissue. Aseptic inflammation was detected in the samples, no demarcation roll from neutrophils was found. Immunohistochemical reaction with antibodies to vimentin detected cells of mesenchymal origin in the tissues adjacent to the scaffold and inside.
CONCLUSIONS:In vitro studies of tissue-engineered tracheal samples detected the scaffold biocompatibility at the cellular level. The presence of a fine connective tissue capsule without manifest fibrosis, no signs of microcirculatory disorders and immune disorders indicate the biocompatibility at the tissue level and absence of toxicity.

Keywords

trachea; 3D-scaffold

Abstract **#734**

PRECISION SURFACE MICROTOPOGRAPHY REGULATES CELL FATE VIA CHANGES TO ACTOMYOSIN CONTRACTILITY AND NUCLEAR ARCHITECTURE

James Carthew[P][1,2], Hazem Abdelmaksoud[3,4], Margeaux Hodgson-Garms[1], Stella Aslanoglou[5,3,6], Roey Elnathan[5,1,3], Joachim Spatz[7,8,9], Juergen Brugger[10], Helmut Thissen[6], Nicolas Voelcker[3,5,6], Victor Cadarso[4,2], Jessica Frith[1]

[1] *Department of Materials Science and Engineering, Monash University, Australia*

[2] *Centre to Impact Antimicrobial Resistance - Sustainable Solutions, Monash University, Australia*

[3] *Melbourne Centre for Nanofabrication, Victorian Node of the Australian National Fabrication Facility.*

[4] *Department of Mechanical and Aerospace Engineering, Monash University, Australia*

[5] *Monash Institute of Pharmaceutical Sciences, Monash University, Australia*

[6] *Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia*

[7] *Department of Cellular Biophysics, Max Planck Institute for Medical Research, Jahnstraße, Heidelberg, Germany*

[8] *Heidelberg University, Institute for Molecular Systems Engineering (IMSE)*

[9] *Max Planck School Matter to Life*

[10] *Microsystems Laboratory, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne 1015, Switzerland*

Corresponding author's email: jessica.frith@monash.edu

Cells can perceive complex mechanical cues from their microenvironment, which in turn influences their development. Although our understanding of these intricate mechanotransductive signals are evolving, the precise roles of substrate microtopography in directing cell fate is still poorly understood. Here we use nanoimprint lithography to generate micropillar arrays ranging from 1 to 10 µm in height, width, and spacing to investigate the impact of microtopography on mechanotransduction. Using this library, we aimed to determine how precise modulation of surface topography can regulate changes in nuclear architecture, chromatin organization, gene expression and cell fate determination. Using Mesenchymal Stromal Cells (MSCs) as a model, we demonstrate how subtle changes to the substrate topography impacts nuclear architecture, leading to prominent DNA re-origination, changes to nuclear envelope protein expression and decreased heterochromatin presence. Additionally, we observe that those micropatterns which stimulated maximal nuclear deformations correlated with the highest degree of osteogenesis, which we demonstrate is dependent on myosin-II-generated tension. We further demonstrate that these effects also translate to the in vivo scenario, in which select micro-topographical features present enhanced cranial wound defect closure. The outcomes of this study determine new insights into nuclear mechanotransduction by demonstrating that force transmission across the nuclear envelope can be modulated by substrate topography, and that this can alter chromatin organization and impacts upon cell fate. These findings have potential to inform the development of microstructured cell culture substrates that can direct cell mechanotransduction and fate for therapeutic applications in both research and clinical sectors.

Abstract **#735**

CONSTRUCTION OF THREE-LAYERED BLOOD VESSELS STRUCTURE BY SHEET-TO-TUBE FOLDING AND EFFECTS OF PERFUSION BIOREACTOR TRAINING ON TISSUE STRUCTURE

Kazutomo Baba[P][1], Andrey Mikhailov[2], Yoshiyuki Sankai[3]

[1] *Graduate School of Systems and Information Engineering, University of Tsukuba, Japan*

[2] *Center for Cybernics Research, University of Tsukuba, Japan*

[3] *Faculty of Engineering, Information and Systems and Center for Cybernics Research, University of Tsukuba, Japan*

Corresponding author's email: baba@golem.iit.tsukuba.ac.jp

Tissue engineered vascular graft (TEVG) have good potential to replace and repair demanded blood vessels. Here, we proposed a novel method to create three-layered TEVG on biocompatible glass fiber scaffolds starting from flat sheet state into tubular shape and to train the resulting tissue by our developed bioreactor system. Constructed tubular tissues were matured and trained under 3 types of individual flow programs, and their mechanical and biological properties were analyzed. The strength of scaffold after cell seeding was 2.83 N which is enough to withstand the pressure of blood flow and the use of sutures. Fluorescent imaging and histological examination of trained vascular tissue revealed that each cell layer has its own individual response to training flow rates. Fluid flow simulation model was created based on experimentally measured tissue geometries; its analysis suggested a correlation between local flow rate fluctuations and fibroblast layer infiltration depth into the scaffold. Concluding: a three-layered tissue structure similar to natural can be created by seeding different cell types in succession, and the following training of the forming tissue with increasing flow by a bioreactor is effective for promoting cell survival, and cell layer formation of desired geometry.

Keywords

Vascular tissue engineering; Bioreactor training; Fluid flow simulation

Abstract **#736**

SOFTER, FASTER, BETTER! – SUBSTRATE MECHANICS DRIVES SUPERIOR SOMATIC CELL REPROGRAMMING OUTCOMES

Justin Cooper-White$_P$[1], Mahfuz Chowdhury[1], Ernst Wolvetang[1], Samuel Zimmerman[2], Jessica Mar[1], Jose Polo[3], Andrew Laslett[4], Christian Nefzger[1,3]

[1] *The University of Queensland*

[2] *Albert Einstein College of Medicine*

[3] *Monash University*

[4] *CSIRO*

Corresponding author's email: j.cooperwhite@uq.edu.au

Substrate mechanics is a well-known modulator of stem cell fate. However, the effect of substrate stiffness on somatic cell reprogramming to induced Pluripotent Stem Cells (iPSC) remains largely unexplored. We assessed the influence of ECM-conjugated polyacrylamide hydrogels of varying stiffness on mouse and human fibroblast reprogramming. Mouse embryonic fibroblasts (MEFs) containing a Dox-inducible reprogramming transcription factor (OKSM) cassette with endogenous Oct4-driven GFP expression were first investigated. Among the hydrogels assessed, a specific stiffness produced up to a 4-fold increase in Oct4 GFP+ miPSC colonies compared to the TCP 'gold standard' control. This hydrogel was then assessed for its efficacy in improving Sendai virus-based human dermal fibroblast reprogramming, confirming that the same hydrogel produced much larger, tighter dome-shaped ALP+ hiPSC colonies compared to the TCPS, and resulted in 10-fold higher yields of hiPSCs. Cell growth, phenotype evolution, and gene expression analysis of cells undergoing reprogramming (by FACS) indicated significant differences in reprogramming kinetics on hydrogels. Time-course RNA-Seq and subsequent bioinformatic analysis revealed differentially-regulated signaling pathways at early and late time points on hydrogels, including many known (but also new!) to reprogramming. This analysis further confirmed that our 'soft' culture environment produced higher quality, more naïve iPSCs faster, offering a novel approach to address some critical deficiencies in current iPSC-manufacturing.

Abstract **#737**

RAPID AND PHYSIOLOGICAL SELF-ASSEMBLY OF MICRO-VASCULAR NETWORKS IN VITRO BY VEGF-DECORATION OF FIBRIN MATRIX

Adelin Rouchon$_P$[1], Priscilla S. Briquez[2], Dirk J. Schaefer[3], Jeffrey A. Hubbell[2], Nunzia Di Maggio[1,3], Andrea Banfi[1,3]

[1] *Department of Biomedicine, University of Basel, Basel, Switzerland*

[2] *Pritzker School of Molecular Engineering, University of Chicago, IL, USA*

[3] *Department of Surgery, Basel University Hospital, Basel, Switzerland*

Corresponding author's email: andrea.banfi@usb.ch

Introduction. Generation of functional vascular networks is an unresolved challenge for 3D engineered tissues. Physiological growth of vascular networks depends on controlled signaling from morphogenic factors like VEGF. Physiological VEGF signaling requires interaction with the extracellular matrix. Here we used an engineered VEGF protein to decorate fibrin hydrogels and rapidly and robustly generate self-assembled micro-vascular networks in vitro.

Methods. Fibrin matrices contained 10mg/ml of human fibrinogen, 3U/ml of thrombin, 3U/ml of factor XIIIa, endothelial cells (HUVEC) and/or adipose stromal cells (ASC) for support. VEGF164 was fused to the transglutaminase substrate peptide NQEQVSPL (TG-VEGF) to enable its covalent cross-linking to fibrin.

Results. Only HUVEC/ASC co-culture generated physiologically differentiated vascular networks, with apico-basal polarization marked by basal laminin and luminal podocalyxin, and open lumens. Vessel formation was influenced by cell density and TG-VEGF: 5x106 cells/ml with 0.1µg/ml of TG-VEGF resulted in a 2.5-fold improvement in vascular density, compared to no VEGF controls, after both 7 and 14 days. Vessel diameters remained similar to controls, with a median of 17µm and therefore compatible with micro-circulation. When injected in vivo, human-derived vascular structures rapidly formed and connected to the host mouse vasculature and were perfused by the systemic circulation, proving their ability to support blood flow.

Conclusions. We identified optimized in vitro conditions, namely 5x106 cells/ml of HUVEC/ASC with 0.1µg/ml of TG-VEGF, to efficiently form a 3D, perfusable, lumenized and physiologically differentiated micro-vascular network within 7 days.

Acknowledgements. This work was funded by the European Union's Horizon 2020 program (grant agreement 801159).

Abstract **#738**

DEVELOPMENT OF NON-VIRAL SCAFFOLD-BASED SYSTEM FOR CONTROLLED DUAL-DELIVERY OF THERAPEUTIC MICRORNA FOR TREATMENT OF LARGE BONE DEFECTS

Joanna M Sadowska[P][1], Monika Ziminska[2], Mark Lemoine[1,3,4], Nicholas Dunne[2,5], Helen O McCarthy[2], Seth Donahue[6], Caroline M Curtin[1], Fergal J O'Brien[1,3,4]

[1] *Tissue Engineering Research Group, Dept. of Anatomy and Regenerative Medicine, RCSI, Dublin, Ireland*

[2] *School of Pharmacy, Queen's University Belfast, Belfast, Northern Ireland*

[3] *Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland*

[4] *Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Dublin, Ireland*

[5] *School of Mechanical and Manufacturing Engineering, Dublin City University, Dublin, Ireland*

[6] *Department of Biomedical Engineering, University of Massachusetts Amherst, USA*

Corresponding author's email: joannasadowska@rcsi.ie

There remains a substantial unmet clinical need for tissue engineered strategies to heal large volume bone defects. The delivery of microRNAs(miRs) induces cells to produce multiple therapeutic proteins with a physiologically relevant release profile and limited side-effects(1-3). Here, we investigate the potential of a scaffold-based approach, for sustained delivery of miRs to enhance bone repair. Collagen-nanohydroxyapatite(coll-nHA) scaffolds(1,2,4) were coupled with a cell-penetrating peptide(5) to establish a miR-activated scaffold system which co-delivers miR-26a-mimic(3) and miR-133a-inhibitor(1,2) for bone repair.

Coll-nHA scaffolds were fabricated using freeze-drying techniques and cross-linked with EDAC/NHS. miRs were complexed with RALA peptide and incorporated into scaffolds. The human mesenchymal stem cells(hMSC) were seeded and evaluated in terms of miR-26a-mimic and miR-133a-inhibitor expression, metabolic activity, DNA content, ALP activity and calcium content. Calcium and H&E staining were evaluated at day 28.

The miRs were homogeneously incorporated into scaffold which retained approx.60% of miR-cargo up to 28 days and transfected the hMSCs. Importantly, the dual-delivery miR-26a-mimic/miR-133a-inhibitor scaffold effectively delivered both cargoes to hMSCs. The miR-activated scaffolds enhanced cellular proliferation of approx. 40%. The miR-26a-mimic and miR-133a-inhibitor scaffolds stimulated ALP activity and the mineralization of hMSCs. The dual-delivery system enhanced osteogenesis to greater extent compared to miR-26a-mimic and miR-133a-inhibitor alone.

Coll-nHA scaffolds were successfully used for delivery of miRs demonstrating potential to be used as next generation of therapeutics for bone regeneration. A novel dual-microRNA scaffold system could be used as a feasible and universal template for delivery of any therapeutic miRs tailoring for a myriad of applications beyond bone repair.

Keywords

Gene delivery; microRNA; Cell-penetrating peptides

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Abstract **#740**

IMMUNOMODULATORY AND PRO-REGENERATIVE EFFECTS OF STRONG PULSED MAGNETIC FIELDS: THE IMPLICATIONS FOR BRAIN REPAIR

Anna Guller[P][1,2,3], Sandhya Clement[2,1], Ewa Goldys[2,1]

[1] *University of New South Wales, NSW, 2032, Australia*

[2] *ARC Centre of Excellence for Nanoscale BioPhotonics, Australia*

[3] *The Institute for Regenerative medicine, Sechenov University, Moscow, 119992, Russia*

Corresponding author's email: a.guller@unsw.edu.au

Brain tissue repair and regeneration represents an exceptional clinical challenge. Acute brain insults such as traumas, strokes, infections, tumors, post-neurosurgery complications as well as chronic neurodegenerative diseases and mental disorders are a major cause of mortality and disability worldwide. Currently available neurorehabilitation strategies required for these patients rely on naturally slow mechanisms as neuroplasticity and adult neurogenesis. We explored an alternative approach to stimulate brain reparation via the rapid effects of non-invasive magnetic stimulation on glial cells. We examined the effects of 18 regimes of the spatially-patterned strong (0.6 – 2 T) pulsed (0.5-50 Hz) magnetic fields (SPMFs) generated by commercially available equipment on human astrocytes and microglial cells cultured in vitro in the conventional and 3D engineered (scaffold-based) models of the brain tissue.

We found that SPMFs can directly control the proliferation and viability of human astrocytes and microglia (frequency- and pulse number-dependent up- and downregulation), selectively modulate the functional polarisation of immune-stimulated microglia with an especially strong shift towards pro-regenerative “M2-like” type in a frequency/intensity-dependent manner. With short exposure to the continuous high-frequency SPMFs, we also achieved ≈70% magnetoporation and the magnetofection of the microglial cells cultured in a monolayer by the commercial iron oxide nanoparticles loaded with GFP plasmid. Finally, with the same SPMF mode, we demonstrated the triggering of the drug release from biocompatible polymer nanoparticles.

Our pioneering findings demonstrate the exceptional potential of SPMFs as a tool for brain repair and regeneration.

Authors acknowledge the support by Sydney Vital Translational Cancer Research Centre.

Abstract **#741**

MORPHOLOGICAL ANALYSES OF TISSUE REACTION TO SUBCUTANEOUS IMPLANTATION OF DECELLULARIZED RAT HEART AND LUNG MATRICES

Alexander Sotnichenko[1], Karina Melkonyan[1], Irina Gilevich[1,2], Alexander Verevkin[1], Yana Kozmay[1], Tatyana Rusinova[1], Denis Ushmarov[1], Sergey Gumenyuk[1], Ilya Bykov[1], Andrey Redko[1], Sergey Alekseenko[1]

[1] *Kuban State Medical University, Krasnodar, Russia*

[2] *Scientific Research Institute - Ochapovsky Regional Clinic Hospital #1, Krasnodar, Russia*

Corresponding author's email: alex24.88@mail.ru

At present time the number of works devoted to the decellularization of various organs has significantly increased. There are many techniques and methods for the preparation of acellular extracellular matrix (ECM), whether based on the physical, chemical and enzymatic effects on the interest.

METHODS:The work was performed on 10 male Wistar rats weighing 210 ± 40 g. Decellularization of the lung and rat heart was performed by modified protocols. The samples were implanted subcutaneously in the interscapular region. Rats were derived from experiment on days 7 and 14. We made a qualitative assessment of the composition of the cellular infiltrate around the implant using immunohistochemistry.

RESULTS:The histological evaluation of rat tissue response to subcutaneous implantation of the decellularized heart matrices was performed. The qualitative cellular composition of the inflammatory infiltrate was studied with an assessment of the dynamic changes in the macrophages, T- and B-lymphocytes amount on days 7 and 14 after the beginning of the experiment.

DISCUSSION & CONCLUSIONS:The results obtained revealed a different tissue response of the recipient organism to ECM implantation. The least expressed was the response to the lung ECM, which successfully integrated into the tissues and did not undergo significant changes. It is shown that the tissue response to implantation depends not only on the quality of decellularization and the efficiency of antigen molecules removal, but also on the initial histological architectonics and quality of preimplantation preparation of the sample.

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Keywords

decellularized matrices; subcutaneous implantation

Abstract **#742**

COMBINATION OF 3D PRINTING AND CASTING FOR MIMICKING NEUROBLASTOMA IN VITRO BONE METASTATIC NICHE

Sanja Aveic$_P$[1,2], Max Seidelmann[1], Simon Janßen[1], Ramin Nasehi[1], Horst Fischer[1]

[1] *Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, 52074 Aachen, Germany*

[2] *Neuroblastoma Laboratory, Pediatric Research Institute Fondazione Città della Speranza, 35127, Padova, Italy*

Corresponding author's email: saveic@ukaachen.de

Metastatic disease remains a leading cause of cancer-related deaths. Metastatic sites are frequently found in cases of neuroblastoma (NB), a malignancy of the developing sympathetic nervous system. Particularly alarming are neoplastic cell invasions of the bone marrow and the bones that strongly correlate with tumor aggressiveness and poor prognosis of patients with NB. In order to improve our current knowledge of metastatic NB biology and improve treatment success, we need appropriate in vitro and in vivo models that more closely approach the native metastatic niche. In this study, we examined how the microstructure of β-tricalcium-phosphate (β-TCP) scaffolds, which contained interconnected microchannels of defined size, affects the interaction of tumor and stromal cells with the extracellular matrix. We confirmed that the tumor microenvironment is dynamically shaped by stroma, which sustain the growth of NB cells inside the metastatic niche. With respect to flat 2D counterparts, cells on 3D β-TCP structures produce more cytokines and define Connexin-43 intracellular localization. Our data confirmed the 3D growth conditions as a determinant factor for the type of cell response to conventional chemotherapy drugs. Together, we confirmed that both, geometry and chemistry, are relevant for translating realistic mechanobiological cues to the metastatic NB. In turn, they assist tumor cells to form spheroid-shaped arrangements facilitating their pro-migratory and pro-invasive patterns.

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Abstract **#743**

HUMAN PLURIPOTENT STEM CELL DERIVED LIMBAL STEM CELLS AS A POTENTIAL TREATMENT MODALITY FOR LIMBAL STEM CELL DEFICIENCY

Heli Skottman[P][1], Meri Vattulainen[1], Maija Kauppila[1], Sebastian Siebelmann[2], Laura Koivusalo[1], Alexander Händel[2], Florian Wabnig[2], Maria Notara[2], Anni Mörö[1], Björn Bachmann[2], Tanja Ilmarinen[1]

[1] *Faculty of Medicine and Health Technology,Tampere University, Finland*

[2] *Department of Ophthalmology, University of Cologne, Germany*

Corresponding author's email: heli.skottman@tuni.fi

Differentiation of corneal limbal stem cells (LSCs) from human pluripotent stem cells (hPSCs) represents an attractive therapeutic option for patients suffering from bilateral LSC deficiency (LSCD). We have previously established an effective production method for clinical grade hPSC-LSCs. Here, our aim is to identify the most potent cell phenotype for corneal tissue engineering and clinical applications.

For the deep phenotyping of the hPSC-LSC populations, quantification of the protein and gene expression studies were conducted. In addition, identity of the cells was further confirmed with whole mount immunohistochemical stainings of human and porcine corneas. Further, the functionality of the identified cell populations where analysed in 3D bioprinted corneal structures, by transplantation to an ex vivo porcine cornea model as well as with the preliminary in vivo studies in LSCD rabbit model.

As a result, we can produce a cell population highly expressing clinically tested corneal progenitor marker p63α/p40 and these cells are able to keep their stemness properties in 3D bioprinting of corneal structures. Interestingly, we also identified an early arising ABCG2-positive cell population and these cells represented a noticeably different LSC-like phenotype as compared to later arising progenitor cell population (ABCG2-negative/p63α-positive). With whole mount immunohistochemical stainings, we confirmed relevant stem cell marker expression in corneal limbal crypts. Furthermore, both ex vivo and in vivo transplantations propose that this newly identified ABCG2-positive cell populations could provide better efficacy in clinical application.

As a conclusion, these two limbal cell populations may represent functionally different stem/progenitor populations with implications in their regenerative efficacy

Abstract **#744**

PEPTIDE HYDROGELS AS DRUG DELIVERY VEHICLES FOR GLIOBLASTOMA

Stephanie Franks$_P$[1], Rita Ferreira[2], Katherine Hannan[2], Ross Hannan[2], David Nisbet[1]

[1] *Laboratory of Advanced Biomaterials, Research School of Electrical, Energy and Materials Engineering, the Australian National University, Canberra, Australia*

[2] *ACRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, the Australian National University, Canberra, Australia*

Corresponding author's email: stephanie.franks@anu.edu.au

Glioblastoma (GBM) is the most common brain tumour in adults[1], but current treatments are limited due to its invasiveness, risk of damage during surgery, and the restricted transport of drugs into the brain[2]. One promising treatment method is the use of biocompatible self-assembling peptide (SAP) hydrogels as localised delivery vehicles. SAPs provide similar properties to the native extracellular matrix (ECM) and are easily administrated[3]. For GBM, they can be implanted post-operatively and provide sustained drug release at the resection site. Here, we investigate SAP-mediated delivery of the anti-cancer drug CX-5461[4].

Fmoc-SAP hydrogels were prepared as described previously, via a pH switch[3]. CX-5461 was encapsulated during assembly, and human GBM cells were cultured on drug-containing gels or with free drug in solution. Target inhibition was determined by RT-qPCR, and cell viability and proliferation were quantified by flow cytometry and live cell imaging.

Stable, shear-thinning hydrogels were formed with and without drug inclusion, with rheology confirming stiffness similar to the brain ECM, and FTIR and CD spectroscopies confirming the expected π-β assembly mechanism. In vitro trials with human GBM cells demonstrated that the two drug groups achieved comparable drug target inhibition and consequent decrease in cell viability at shorter timepoints. However, a significantly prolonged effect (>7 days) was observed for the drug-loaded SAPs compared to the free drug alone.

These programmable SAP hydrogels may improve therapeutic outcomes in GBM by increasing local drug accumulation and efficacy, while also providing easy implantation, high biocompatibility, and helping to support surrounding healthy tissues.

Keywords

Drug delivery

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Abstract **#745**

MULTI-DIMENSIONAL CELLULAR MODELS MIMICKING THE HETEROGENEITY OF OSTEOSARCOMA STEM CELL NICHE FOR INCREASING THE PREDICTIVE POTENTIAL OF THE IN VITRO STUDIES

Monica Montesi[1], Silvia Panseri[1], Elisabetta Campodoni[1], Monica Sandri[1], Giada Bassi[1]
[1] *Institute of Science and Technology for Ceramics - National Research Council of Italy (ISTEC-CNR)*
Corresponding author's email: monica.montesi@istec.cnr.it

Worldwide cancer remains the second-most common cause of death, despite the advances in prevention, early detection, treatment; among the bone cancer Osteosarcoma (OS) is the most common type diagnosed especially in children and young adults1.
The failure of the osteosarcoma conventional therapies leads to the growing need for novel therapeutic strategies. The lack of specificity for the Cancer Stem Cells (CSCs) population has been recently identified as the main limitation in the current therapies2. Moreover, the traditional two-dimensional (2D) in vitro models, employed in the drug testing and screening as well as in the study of cell and molecular biology, are affected by a poor in vitro-in vivo translation ability3. To overcome these limitations, this work provides two "tumour engineering" approaches as new tools to address osteosarcoma and improve therapy outcomes. In detail, two different hydroxyapatite-based bone-mimicking scaffolds4,5 were used to recapitulate aspects of the in vivo tumour microenvironment, focusing on CSCs niche. The biological performance of human osteosarcoma cell lines (MG63 and SAOS-2) and enriched-CSCs were deeply analysed in these complex cell culture models. The results highlight the fundamental role of the tumour microenvironment proving the mimicry of osteosarcoma stem cell niche by the use of CSCs together with the biomimetic scaffolds, compared to conventional 2D culture systems. These advanced 3D cell culture in vitro tumour models could improve the predictivity of preclinical studies and strongly enhance the clinical translation, with the ultimate goal to be applied in personalized medicine.

Keywords
Tumour engineering; Canser Stem Cells; 3D in vitro model

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Abstract **#747**

ELUCIDATING THE INFLUENCE OF LYSOSOMAL STRESS ON SCHWANN CELLS

Karen Libberecht$_P$[1,2], Tim Vangansewinkel[1,2], Ivo Lambrichts[1], Ludo Van Den Bosch[2], Esther Wolfs[1]

[1] *Faculty of Medicine and Life Sciences, BIOMED, Hasselt University, Diepenbeek, Belgium.*

[2] *Laboratory of Neurobiology, VIB, Center for Brain and Disease Research, Leuven, Belgium*

Corresponding author's email: karen.libberecht@uhasselt.be

INTRODUCTION: Endo-lysosomal-autophagy alterations have been demonstrated to play an important role in neurodegenerative diseases1. In myelinating cells this pathway is crucial for transporting newly synthesized myelin proteins towards the plasma membrane. This renders myelinating cells to be particularly sensitive for lysosomal stress and autophagic failure, resulting in altered myelinating activity as seen in Charcot-Marie-Tooth disease2. However, the role of lysosomal dysfunction in defective myelinating cells and neurodegeneration remains poorly understood.

Here, we aim to mimic the effect of lysosomal stress on the peripheral myelinating cells: Schwann cells (SC). Isolation of SC is challenging and the use of clinically relevant animal models is limited in neurodegenerative diseases3. Therefore, an alternative is needed to elucidate underlying pathomechanisms. Here we aim to use human dental pulp stem cells (DPSC) as an alternative, as they can be differentiated towards SC-like cells (DPSC-SC)4.

METHODS: Lysosomal damage was induced in DPSC-SCs using chloroquine in different concentrations and incubation times. Lysosomal alterations and membrane permeabilization were visualized using Lysotracker Deep red® live imaging and a GAL3-LAMP1 puncta-assay, respectively. To elucidate the influence of lysosomal enzyme activity upon lysosomal stress, a Cathepsin B activity assay was performed. Finally, cell viability was monitored using Alamar blue and PI assays.

DISCUSSION & CONCLUSIONS: Our data indicate a dual effect of chloroquine on the lysosomal integrity of DPSC-SC, and a concentration-dependent loss of cell viability.

Further research will compare the lysosomal integrity in healthy stem cells after chloroquine incubation versus stem cells derived from patients with Charcot-Marie-Tooth disease.

Keywords

Autophagy; Human Dental pulp stem cells; Peripheral nerve disease

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Abstract **#748**

EVALUATION OF DIFFERENT PLACENTAL EXTRACELLULAR MATRIX INJECTABLE HYDROGELS FOR CARDIAC TISSUE REPAIR

Karl H Schneider[P1,2], Gabriela Eder[2], Diego Aguilar[1], Sabrina Rohringer[1], Patrick Haider[3], Herbert Kiss[4], Andreas H Teuschl[5], Bruno K Podesser[1,2,6], Attila Kiss[1,2], Helga Bergmeister[1,2,6]

[1] *Center for Biomedical Research, Medical University of Vienna, Austria*

[2] *Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria*

[3] *Division of Cardiology, Department of Medicine II, Medical University of Vienna*

[4] *Department of Obstetrics and Gynecology, Division of Fetomaternal Medicine, Medical University of Vienna, Vienna*

[5] *University of Applied Sciences, FH Technikum Vienna*

[6] *Austrian Cluster for Tissue Regeneration*

Corresponding author's email: karl.schneider@meduniwien.ac.at

Hydrogels, made from biological tissues, are gaining more and more importance in tissue engineering and regenerative medicine due to their natural composition and broad applicability. Beside in vitro application for 3D cultures or as a surface coating, recent preclinical and clinical studies demonstrated that injectable materials facilitate functional tissue remodeling in wounds and necrotic tissues. However, the underlying protective mechanisms are largely unknown, although the modulating effect of hydrogels on immune cells during injury and repair may play a role.

The use of human placental tissue was approved by the ethics committee of the Medical University of Vienna (EC No. 1602/2018). ECM hydrogels were produced from different parts of the human placenta and porcine cardiac tissue by decellularization, followed by an enzymatic digestion for solubilization of the ECM matrix. ECM hydrogels were characterized thoroughly to be compared to each other in molecular composition, immunogenicity and biomechanics. Cytocompatibility was investigated by performing cell seeding experiments on ECM hydrogel coated tissue culture plates. ECM hydrogels showed low DNA residues and preservation of cell interacting and structural ECM proteins. Rheology revealed clot formation of ECM hydrogels in reproducible biomechanical strength dependent on respective hydrogel type and concentration. All tested ECM hydrogels supported cell proliferation and survival of human endothelial cells, fibroblasts and smooth muscle cells. Human macrophage cultures showed reduced survival rates by ECM-hydrogels in vitro. First in vivo experiments to investigate the promotion of cardiac tissue repair in respect to infarct healing and remodeling in association with inflammation are ongoing.

Abstract **#749**

SYNTHESIS AND CHARACTERIZATION OF MULTICOMPONENT HYDROGELS COMPOSED OF GELATIN-POLY (METHACRYLIC ACID) DOUBLE NETWORK AND NANO-PARTICLES OF HYDROXYAPATITE AND B - TRICALCIUM PHOSPHATE FOR BONE TISSUE ENGINEERING APPLICATIONS AND CONTROLLED DELIVERY OF OXAPROZIN DRUG

Vukasin Ugrinovic[P][1], Pavle Spasojevic[2,1], Bojan Bozic[3], Rada Petrovic[4], Djordje Janackovic[4], Vesna Panic[1], Djordje Veljovic[4]

[1] *Innovation Center of the Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia*

[2] *Faculty of Technical Sciences, University of Kragujevac, Svetog Save 65, 32000 Cacak, Serbia*

[3] *Institute of Physiology and Biochemistry „Ivan Đaja“, Faculty of Biology, University of Belgrade, Serbia*

[4] *Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia*

Corresponding author's email: djveljovic@tmf.bg.ac.rs

Scaffolds are three-dimensional porous structures designed to, temporally or permanently, replace a critical bone volume loss, and act as a structural support for adhesion, proliferation and differentiation of osteoblasts, which is the main precondition for healing and regeneration of damaged tissue. For this purpose, hydrogels are particularly attractive due to high porosity, swelling ability, structural similarity to extracellular matrix and potential drug deliverability. However, the soft nature and lack of bioactivity limits its application in bone tissue engineering (BTE). In this study we synthesized composite hydrogels based on double network of gelatin and poly(methacrylic acid) (PMAA), and nano-particles of hydroxyapatite (HA) and β - tricalcium phosphate (β−TCP) as a reinforcement phases. Gelatin-PMAA double network is composed of covalently crosslinked PMAA network and physically crosslinked gelatin, and is characterized by improved mechanical and biological properties in comparison to single network hydrogels. Moreover, the incorporation of nano-sized inorganic fillers HA and β−TCP, created bone-mimicking structure with optimized mechanical and biological properties. The optimization of mechanical and morphological properties, as well as bioactivity, was performed by variation of filler content and HA/ β−TCP ratio. Composites were characterized by FTIR, SEM, mechanical testing, MTT assay and in vitro bioactivity. The loading and release of anti-inflammatory drug oxaprozin was also investigated in vitro. SEM images revealed macroporous structure and compressive strength values confirmed favorable mechanics of the obtained composite scaffolds. The increase of HA content led to improve physical-mechanical properties, while increase of β−TCP favored bioactivity and cell metabolic activity.

Abstract **#750**

SEMAPHORIN3A COUPLES OSTEOGENESIS AND ANGIOGENESIS IN TISSUE-ENGINEERED OSTEOGENIC GRAFTS

Andrea Banfi[P][1,2], Andrea Grosso[1], Priscilla S. Briquez[3], Dirk J. Schaefer[1,2], Jeffrey A. Hubbell[3], Nunzia Di Maggio[1]

[1] *Department of Biomedicine, University of Basel, Basel, Switzerland*

[2] *Department of Surgery, Basel University Hospital, Basel, Switzerland*

[3] *Pritzker School of Molecular Engineering, University of Chicago, IL, USA*

Corresponding author's email: andrea.banfi@usb.ch

Coupling of angiogenesis and osteogenesis is crucial to generate vascularized bone grafts. Semaphorin3a regulates bone formation through the Neuropilin-1 receptor (Nrp1). We previously found that Vascular Endothelial Growth Factor (VEGF) dose-dependently: 1) impairs osteogenesis by increasing bone resorption; 2) inhibits endothelial Sema3a expression in muscle. Here we investigated whether: a) VEGF impairs bone formation by inhibiting endogenous Sema3a expression; b) Sema3a treatment could couple angiogenesis and osteogenesis in engineered bone grafts.

VEGF or Sema3a proteins were engineered with a transglutaminase substrate peptide (TG-VEGF and TG-Sema3a) to allow cross-linking into fibrin hydrogels. Osteogenic grafts were prepared with human BMSC and hydroxyapatite granules in fibrin hydrogels containing TG-VEGF, TG-Sema3a or both (ratio 1:1) and implanted ectopically in nude mice. Sema3a/Nrp1 signaling was blocked with a specific antibody.

High TG-VEGF caused severe bone loss and downregulation of Sema3a expression. Low TG-VEGF preserved both bone formation and Sema3A expression. Blocking of Sema3a/Nrp1 signaling: 1) impaired bone formation, increasing osteoclasts recruitment and reducing human progenitor survival; 2) decreased vascular invasion; and 3) impaired endogenous Sema3a expression, recruitment of Nrp1-expressing monocytes and phospho-SMAD2/3 activation in both human progenitors and host endothelium, consistently with a positive feed-back loop we previously described between Sema3a and TGF-β1.

Conversely, TG-Sema3a co-delivery prevented bone loss by high TG-VEGF, while preserving vascular growth. TG-Sema3a alone (without TG-VEGF) significantly promoted both bone formation and vascularization.

These data show that Sema3a is a key molecular link coupling angiogenesis and bone formation, and a promising target to generate vascularized bone grafts in a clinical setting.

Abstract **#752**

AGE-DEPENDENT CHANGES IN PERIVASCULAR STEM CELLS AND THE IMPACTS OF ALTERED SENSING ON VASCULAR AGEING

Justin Cooper-White[P1], Mahfuz Chowdhury[1], Ernst Wolvetang[1], Desi Veleva[1]

[1] *The University of Queensland*

Corresponding author's email: j.cooperwhite@uq.edu.au

Perivascular niches exist throughout all tissues, forming an interconnected and highly responsive 'maintenance and repair' system across our body, and their decline in function is central to many age-related diseases. Perivascular stem cells (or pericytes, PCs) within these niches are expected to be exquisitely more sensitive to changes in their local microenvironment than other tissue-resident cells. However, little is understood as to what intrinsic changes these cells undergo with ageing and how this affects their ability to maintain the niche and support tissue repair. This work aimed to investigate the intrinsic changes in human pericytes as a function of age, and thereafter the impacts of any changes on their function within an in vitro perivascular niche model. We show that fat pad-derived adult pericytes exhibit minimal changes in gold-standard phenotype markers or morphological traits with increasing age, but show substantial changes in global gene expression, protein and growth factor production. Functionally, PCs change their extent and types of interactions with endothelial cells (ECs) as they age, but in contrast, are more 'activated' and 'angiogenic', based on their secretome profile. We show that the addition of pro-angiogenic factors was insufficient to overcome these functional deficits, and moreover, that these changes are a result of altered cell-cell communication and mechano-signalling. These results provide new insight into how age-related changes in pericyte function may contribute to increased refraction of microvessel networks as we age, and suggest a number of new options for therapeutic targeting to potentially reduce age-related decline in perivascular niche function.

Abstract **#753**

DESIGN OF IN VITRO CULTURE SYSTEM USING MELT ELECTROWRITING

Ezgi Bakirci$_P$[1], Andrei Hrynevich[1], Natascha Schaefer[2], Dieter Janzen[2], Annalena Wieland[3], Ouafa Dahri[1], Jaehyeon Kim[1], Estaban Ceballos[1], Pamela L. Strissel[3], Reiner Strick[3], Carmen Villmann[2], Paul D. Dalton[1]

[1] Department of Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, University Hospital of Würzburg, Pleicherwall 2, 97070, Würzburg, Germany

[2] Department of Clinical Neurobiology, University Hospital of Würzburg, Versbacherstr. 5, 97078 Würzburg, Germany

[3] Department of Gynecology and Obstetrics, Laboratory for Molecular Medicine, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, University Str. 21-23, 91054 Erlangen, Germany

Corresponding author's email: ezgi.bakirci@fmz.uni-wuerzburg.de

Three dimensional (3D) in vitro culture systems are important to mimic the heterogeneous complexity of human tissues [1]. The development of additive manufacturing technologies allows to fabricating accurate and precise in vitro models [2]. Here we focused on melt electrowriting (MEW) which serves an excellent control of fiber resolution and helps to mimic the heterogeneous complexity of native tissues in synthetic scaffolds.

The capacity to fabricate complex and microscale structures using MEW provides new opportunities in in vitro culture systems. Three types of MEW designs were investigated; 1) reinforcing matrices, 2) guiding structure, 3) migration assays (competitive assays). MEW structures printed with poly(ε-caprolactone) (PCL) (Purasorb PC-12) were used in different in vitro models stated above.

Lattice scaffolds were utilized for reinforcement purposes increasing the compressive modulus of ultra-soft hydrogel to allow manipulation during culture. A migration assay was established, where the presence of various chambers enabled a 3D competitive cell migration assay. Furthermore, peptide coated scaffolds were used as a model for studying the impact of the design and dimensions on glioblastoma cells and astrocytes. The coating of MEW scaffolds were developed based on six-arm star-shaped NCO-poly(ethylene oxide-stat propylene oxide) (sP(EO-stat-PO)) [3]. The IKVAV and RGD peptides were linked covalently to MEW fibers through a reaction with isocyanates. The results demonstrate that the coating improved cell attachment and enhanced biomimicry of native ECM.

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Abstract **#754**

MECHANO-REGULATED PROTEINS IN PATHOLOGICAL HEART

Stefania Pagliari[1,2], Vladimir Vinarsky[1,2], Fabiana Martino[1,2,3], Ana Rubina Perestrelo[1,2], Jorge Oliver-de-la-Cruz[1,2], Jan Vrbsky[1,2], Guido Caluori[1], Giancarlo Forte[1,2]

[1] *International Clinical Research Center (ICRC) of St Anne's University Hospital, CZ-65691 Brno, Czech Republic*

[2] *Competence Center for Mechanobiology in Regenerative Medicine, INTERREG ATCZ133, CZ-62500 Brno, Czech Republic*

[3] *Faculty of Medicine, Department of Biology, Masaryk University, CZ-62500 Brno, Czech Republic*

Corresponding author's email: stefania.pagliari@fnusa.cz

Cardiac remodeling entails the modification of ventricle 3D architecture as a result of the deposition of de novo synthesized non-compliant extracellular matrix (ECM). While the impact of the biochemical changes associated with pathological ECM deposition has been described in animal models, the basis and impact of structural changes due to collagen assembly and alignment in heart failure (HF) are poorly described. We combined SEM, AFM and 3D two-photon microscopy imaging, with the aim of characterizing the alterations occurring in human cardiac ECM biophysical properties at end-stage HF. By this approach, we generated a comprehensive, high-resolution map of the modifications occurring in the collagen network organization during pathological cardiac ECM remodelling.Next, we adopted a multi-omics approach coupled to bioinformatics analysis to identify novel mechanosensitive proteins whose function is perturbed in pathological heart. We demonstrate the reactivation of mechanosensitive YAP in the pathological heart occurs in a cell-specific fashion, thus contributing to the establishment of fibrosis, while being involved in the increased contractility of hypertrophic cardiomyocytes.More importantly, we provide evidence that the localization and function of RNA-binding proteins can be controlled by the increased biomechanical stress generated by cardiac ECM remodelling and propose their role as mechanosensitive switches affecting RNA splicing and maturation in the pathological heart. Altogether, we identify the dysregulation of known and novel mechano-regulated proteins in the heart, thus defining the mechano-pathological pathways controlling cell contractility and RNA homeostasis to shape cardiac cell response to the ensuing biomechanical stress caused by ECM pathological remodelling.

Abstract **#755**

GLYCAN RECEPTOR TARGETING WITH BIO-CONJUGATED NANODIAMONDS IN CENTRAL NERVOUS SYSTEM: VALIDATION IN 3D BRAIN SCAFFOLD-BASED TISSUE MODELS

Mina Ghanimi fard[1,2,3], Nicole Cordina[1], Anna Guller[4,5,6], Nicki Packer[1,2,3], Lindsay Parker[1,2,3]

[1] *Department of Molecular Sciences Macquarie University*

[2] *Centre for Nanoscale BioPhotonics Macquarie University*

[3] *Sydney Vital Translational Cancer research institute*

[4] *The Graduate School of Biomedical Engineering University of New South Wales (UNSW)*

[5] *Centre for Nanoscale BioPhotonics University of New South Wales*

[6] *The Institute for Regenerative medicine, Sechenov University, Moscow, 119992, Russia*

Corresponding author's email: mina.ghanimi-fard@hdr.mq.edu.au

Glycosylation is a post-translational modification that attaches glycan receptors to cell surface proteins and lipids. Glycans are a major component of the extracellular matrix and are highly important in cell-cell interactions. Abnormal glycosylation is evident during multiple chronic brain pathologies including brain cancer, making glycan receptors excellent biomarkers for selective targeting of affected cells. Lectin proteins are widely used to identify many glycan residues. Here, we investigated in vitro targeting of sialic acid (wheat germ agglutinin), fucose (Aleuria aurantia lectin), and N-acetylglucosamine (tomato lectin) glycan moieties in glioblastoma astrocytes, normal neurons and microglia cells cultured in conventional monolayers and in 3D brain scaffold-based organ-specific models.

We bio-conjugated three different types of lectin proteins to 120-nm fluorescent nanodiamond particles with nitrogen vacancy centres. The binding of conjugated nanodiamonds to the glycan receptors of astrocytes, neurons and microglia cells in standard 2D cell cultures was evaluated. Next, we developed tissue engineered constructs combining the decellularized brain scaffolds and the brain cells of choice a 3D culture system, including the unique brain scaffold-based immunoids containing microglia cells. These models helped us better assess cell targeting in each cell type in a biologically accurate models reconstructing natural-like microenvironments.

In 2D and 3D cell cultures we found that Aleuria aurantia lectin, targeting core fucose glycan receptors, binds to glioblastoma cells with the highest affinity. Our findings indicate that the bio-conjugated nanodiamonds developed in this study could be used in future studies as a drug delivery vehicle for targeting human brain cancer and immune cells.

Abstract **#756**

HUMAN AMNIOTIC MEMBRANE TENSION CRITICALLY IMPACTS MITOCHONDRIAL FUNCTION AND CELL VIABILITY IN VITRO

Laura Poženel[P][1,2], Andrea Lindenmair[3,2], Katy Schmidt[4], Andrey V. Kozlov[1,2], Johannes Grillari[1,2,5], Susanne Wolbank[1,2], Asmita Banerjee[1,2], Adelheid Weidinger[1,2]

[1] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingenstraße 13, 1200 Vienna, Austria

[2] Austrian Cluster for Tissue Regeneration, Vienna, Austria

[3] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Garnisonstraße 21, 4020 Linz, Austria

[4] Medical University of Vienna, Center for Anatomy and Cell Biology, Division of Cell and Developmental Biology, Schwarzspanierstraße 17, 1090 Vienna, Austria

[5] University of Natural Resources and Life Sciences Vienna, Department of Biotechnology, Muthgasse 18, 1190 Vienna, Austria

Corresponding author's email: laura.pozenel@trauma.lbg.ac.at

The human amniotic membrane (hAM) is successfully used for therapeutic applications, yet mostly in a decellularized form. Since amniotic cells display stem cell characteristics, recent research has initiated a paradigm shift towards the use of hAM with its original viable cell population. However, sustaining cellular viability of hAM is challenging. It has been suggested that in vivo, the apoptosis of amniotic cells is connected to loss of tissue tension. Therefore, our study addressed the question whether tissue distention impacts the cellular viability of hAM in vitro and whether mitochondria play a critical role in this process.

hAMs biopsies were incubated for 7–21 days, either distended on CellCrown(TM) inserts, or non-distended. In non-distended hAMs B-cell lymphoma 2-associated X protein (BAX) and B-cell lymphoma (BCL)-2 ratios were increased at day 7, followed by increased caspase 3 expression at day 14. At day 21, decrease of mitochondrial respiration and ATP was observed and consequently, loss of cell viability. In distended hAMs, mitochondrial function and cell viability were largely maintained.

Our data suggest that tissue distention prolongs the life span of hAM cells in vitro. Loss of tissue tension leads to an unknown tension-driven mitochondrial pathway (TDMP), resulting in mitochondria impairment, induction of caspase 3-mediated apoptosis and loss of viability of hAM cells. Further exploration of this mechanism will bring more understanding in the hAM physiology and support the optimization of hAM tissue cultures.

The study was funded by the Austrian Research Promotion Agency (FFG grant number 867674).

Abstract **#757**

DESIGN OF INJECTABLE AND SELF-HEALING BORONIC ACID-BASED HYDROGELS FOR A MATERIAL-ASSISTED CELL THERAPY OF OSTEOARTHRITIS

Nathan Lagneau[P][1], Pierre Tournier[1], Boris Halgand[2], François Loll[1], Jérôme Guicheux[1,2], Catherine Le Visage[1], Vianney Delplace[1]

[1] Université de Nantes, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

[2] Université de Nantes, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

Corresponding author's email: nathan.lagneau@univ-nantes.fr

Intra-articular injection of Mesenchymal Stromal Cells (MSCs) has been recently proposed as a promising treatment for osteoarthritis (OA); yet, cell therapies still suffer from limitations (e.g., cell death upon injection, cell leakage). To overcome these drawbacks, MSCs could be encapsulated in injectable, cytoprotective hydrogels to support their viability and bioactivity. However, for successful intra-articular delivery, hydrogels should also be able to bear loads and absorb shocks. In this context, we envisioned to develop new, injectable and self-healing, boronic acid-based hydrogels to improve OA cell therapy.

Polysaccharides were first successfully functionalized with either boronic acid derivatives or diol-containing molecules via single-step syntheses, as confirmed by NMR or elemental analysis. A vast screening of boronic acids and diols led to the discovery of an innovative couple that allows the instantaneous formation of dynamic hydrogels under physiological conditions of pH and temperature, and is applicable to a variety of polysaccharides (e.g., hyaluronic acid, alginate, carboxymethyl cellulose). These hydrogels are stable for at least 2 months under physiological conditions with a shear elastic modulus ranging from 100 Pa to 1000 Pa. Due to their dynamic nature, the resulting hydrogels are injectable and can self-heal instantly after network disruption, making them suitable for intra-articular applications.

MSCs encapsulated in the newly designed hydrogels displayed excellent viability (> 90%) after 7 days of culture. The evaluation of the paracrine activity of encapsulated MSCs, as a function of hydrogel composition and mechanical properties, is now under investigation, laying the foundations for a new generation of OA cell therapy.

Abstract **#758**

COACTIVATION OF ENDOGENOUS WNT10B AND FOXC2 BY CRISPR ACTIVATION ENHANCES BMSC OSTEOGENESIS AND PROMOTES CALVARIAL BONE REGENERATION

Mu-Nung Hsu[P][1]

[1] *Department of Chemical Engineering, National Tsing Hua University*

Corresponding author's email: peter5552345@hotmail.com

CRISPR toolbox has been wildly expanded nowadays to accomplish precise, robust and easy genetic manipulation, but its potential for tissue regeneration has yet to be fully explored. The nuclease-dead Cas9 (dCas9) proteins are programmable transcriptional regulators that can be reconstructed with transcriptional activation domains for multiplexing gene regulation[1]. To promote BMSC differentiation toward osteogenesis and improve calvarial bone healing by BMSCs, we constructed a hybrid baculovirus vector (Bac-CRISPRa) that harbored a synergistic activation mediator (SAM)-based CRISPRa system to activate Wnt10b (that triggers the canonical Wnt pathway)[2] and forkhead c2 (Foxc2) (that elicits the noncanonical Wnt pathway)[3] in BMSCs. qRT-PCR revealed that Bac-CRISPRa enabled CRISPRa delivery and potently activated endogenous Wnt10b and Foxc2 expression in BMSCs for >14 days. Furthermore, the robust and prolonged coactivation of both Wnt10b and Foxc2 additively enhanced osteogenic differentiation while inhibiting adipogenic differentiation of BMSCs. Implantation of CRISPRa-engineered BMSCs into the calvarial defects remarkably improved the bone healing in rats. These data underscore the potentials of CRISPRa system for tissue engineering.

Keywords

CRISPRa

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Abstract **#759**

EFFECT OF BIOMATERIAL COMPOSITION ON MSC CHONDROGENESIS EMBEDDED IN A HYALURONAN COMPOSITE CONTAINING COLLAGEN FIBRILS

Andrea Schwab[P][1], Flurina Staubli[1], David Eglin[1], Matteo D'Este[1]

[1] *AO Research Institute, Davos*

Corresponding author's email: andrea.schwab@aofoundation.org

Biomimetic properties of biomaterial-inks, induced via 3D-bioprinting, allow mimicking the architecture of microfibrillar components in extracellular matrix 1. Aim of this study was to investigate the effect of collagen (col) concentration and col source in combination with a HA biomaterial on hMSC chondrogenesis.

Tyramine-modified HA was mixed with col from rat tail or from Jellyfish (J) (HA–col in mg/ml): 12.5-2.5 (1:1) and 16.6–1.7 (2:1). hMSC micropellets were embedded into biomaterials (5 mio/ml), enzymatically crosslinked for 3D-bioprining (3D Discovery RegenHu) or casting and light-crosslinking. hMSC pellets served as control group. Samples were cultured in chondrogenic media containing TGF-β1 (21 days) and analyzed for cartilaginous matrix synthesis (histology, proteoglycan quantification, PCR).

hMSCs migrated throughout biomaterial and differentiated towards chondrogenic lineage in HA-col marked by increased proteoglycan content (5000ugGAG/ugDNA), which was not observed for HA and HA-J. Upregulation of cartilage markers col II and aggrecan and SOX9 (100/300; 50/100; 4/4 fold-change) confirmed MSCs differentiation in HA-col (2:1/1:1), accompanied by little upregulation of col-I (0.6/0.6 fold-change), RunX2 (<2/2 fold-change) and col X that was more pronounced in pellet control (15 fold-change) compared to HA-col. hMSC embedded in HA and HA-J had low expression of cartilage markers. Col fibrilswith anisotropic orientation after 3D-bioprinting was shown by fluorescence imaging. Micropellets showed deformation in circularity in HA-col (0.96±0.02) upon printing (0.81±0.13, 18G needle) .

HA-col composite has proven to support hMSC migration and chondrogenesis with stronger chondrogenic response at higher col content. Shear induced col fiber orientation of THA-col bioink allows to control and direct cell migration.

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Abstract **#760**

3D SCREENING PLATFORM OF CELL-ADHESION PEPTIDES: GOING BEYOND RGD

Aurelien Forget$_P$[1]

[1] *Institute for Macromolecular Chemistry*

Corresponding author's email: aurelien.forget@makro.uni-freiburg.de

Cell-adhesion peptides (CAPs) such as the ubiquitous arginine-glycine-aspartic acid (RGD) sequence have allowed the functionalization of synthetic materials to mimic macromolecules of the extracellular matrix (ECM). However, the variety of ECM macromolecules makes it challenging to reproduce all of the native tissue functions with only a limited variety of CAPs. Screening of libraries of CAPs, analogous to high-throughput drug discovery assays, can help to identify new sequences directing cell organization. Challenges to this approach include the automation of cell seeding in three dimensions and characterization methods.[1]

Here, we report a method for robotically generating a library of 16 CAPs to identify microenvironments capable of directing a chain-like morphology in olfactory ensheathing cells (OECs), a cell type of particular interest for spinal cord injury to guide axon growth. This approach resulted in the identification of two CAPs not previously reported to interact with OECs to direct their morphology into structures potentially suitable for axon guidance.[2]

Following a similar approach, we are extending the screening to a broader variety of cell lines. With this new work, we are now equipped to better understand the cell interactions with the ECM. Screening of cancer cell lines will help us to investigate the progression of cancer as a function of the ECM biological adhesive properties and ECM mechanical properties Similar screening with mesenchymal stem cells will help us to discover new CAPs responsible for directing the organization of cells into structures pertinent for tissue regeneration.

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Abstract **#762**

NOVEL SENOLYTICS BASED ON ARACHIDONIC ACID METABOLISM

Ingo Lämmermann$_P$[1], Vera Pils[1], Marie Narzt[2], Lucia Nanic[3], Ivica Rubelj[3], Anja Wilmes[4,5], Paul Jennings[4,5], Florian Gruber[6], Johannes Grillari[1,2]

[1] Institute of Molecular Biotechnology, Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

[2] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre, Vienna, Austria

[3] Institut Ruđer Bošković, Laboratory for Molecular and Cellular Biology, Zagreb, Croatia

[4] Division of Molecular and Computational Toxicology, Amsterdam Institute for Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

[5] Division of Physiology, Department of Physiology and Medical Physics, Medical University of Innsbruck, Innsbruck, Austria

[6] Division for Biology and Pathobiology of the Skin, Department of Dermatology, Medical University of Vienna, Vienna, Austria

Corresponding author's email: ingo.laemmermann@boku.ac.at

Pharmacological compounds that selectively eliminate senescent cells and are termed "senolytics" were found to extend the healthspan of mice, to prevent or delay the occurrence of senescence-associated diseases and the development of frailty.

Here we discovered an altered lipid metabolism in senescent human dermal fibroblasts (HDF), which was centered on an elevated phospholipase A2 (PLA2) activity generating elevated levels of intracellular lysophosphatidylcholines (LysoPCs) and arachidonic acid (AA). These LysoPCs were significantly elevated in replicative senescence (telomere dependent) as well as in doxorubicin-induced premature senescence (SIPS; telomere independent) and correlated well with the senescence markers p21 and SA-β-galactosidase activity.

High intracellular levels of AA can induce apoptosis, which is usually prevented by conversion of AA into eicosanoids by cyclooxygenases (COX) and lipoxygenases (ALOX) or by reacylation and degradation processes after activation of AA by acyl-CoA synthases (ACSL). By blocking AA conversion with inhibitors against COX, ALOX and ACSL we achieved a senolytic effect in SIPS HDF, as well as lung fibroblasts and renal epithelial cells, where the EC50 value for senescent cells was up to 700-fold lower for senescent cells compared to quiescent control cells. The senolytic effect could be rescued with either an activator or inhibitor of cPLA2 and was thereby confirmed to be dependent on the elevated PLA2 activity of senescent cells. In line with previous reports, AA-induced apoptosis was ATP sensitive suggesting the involvement of ADP/ATP translocases.

Taken together, our data suggests, that the inhibition of AA conversion is a promising strategy for the development of senolytic therapies.

Abstract **#763**

LONG-TERM LOW-MAGNITUDE FLUID SHEAR STRESS DIRECTS BONE MARROW MESENCHYMAL STEM CELLS TOWARD OSTEOGENIC LINEAGE

Shuntaro Yamadaᵽ[1], Mohammed A Yassin[1], Jan Hansmann[2,3], Kamal Mustafa[1]

[1] *Department of Clinical Dentistry, Faculty of Medicine, University of Bergen*

[2] *Translational Center Regenerative Therapies, Fraunhofer Institute for Silicate Research ISC*

[3] *Chair of Tissue Engineering and Regenerative Medicine, University Hospital Wuerzburg*

Corresponding author's email: shuntaro.yamada@uib.no

Growing evidence indicates that the modulation of environmental factors alone is sufficient to regulate mesenchymal stem cells (MSC) fate to certain lineages although differentiation is commonly induced chemically in vitro. When it comes to clinical translation, however, it is to be induced without chemical treatment in order to overcome concern over unexpected adverse impacts. Recently, we have developed a perfusion bioreactor specifically for bone tissue engineering where MSC on three-dimensional scaffolds receive controlled fluid shear stress (FSS) for long term. In this study, by applying the bioreactor, biological responses of MSC to FSS including osteogenic differentiation were evaluated. MSC from Lewis rat bone marrow (rBMSC) were seeded on microporous scaffolds of a synthetic copolymer, Poly(L-lactide-co-trimethylene carbonate), and were subject to low-magnitude FSS (approximately 1.0-3.5e-4 Pa) up to 21 days. FSS induced by the system was characterised by an in-silico model. While FSS supressed cell proliferation, the expression of early and late osteogenic markers including Runt-related transcription factor 2, Alkaline phosphatase, Osterix, Osteopontin and Osteocalcin were significantly upregulated by FSS over the period of 21 days compared to a static counterpart. Similarly, mineral formation was observed on day 21 in the FSS group. Interestingly, FSS induced the upregulation of a definite and direct gene target of canonical Wnt signalling pathway, Axin2, which suggests the active role of the pathway in FSS-induced osteogenesis. Taken together, we have demonstrated that long-term low-magnitude FSS alone can direct the rBMSC fate toward osteogenic lineage, which can potentially improve the efficiency of bone regeneration after transplantation.

Abstract **#764**

B-SHEET FORMING PEPTIDE HYDROGELS: FROM SELF-ASSEMBLY TO FUNCTIONAL BIOMATERIALS

Alberto Saiani[P][1,2], Aline F Miller[3,2,4]

[1] *University of Manchester, Department of Materials, UK*

[2] *University of Manchester, Manchester Institute of Biotechnology, UK*

[3] *University of Manchester, Department of Chemical Engineering and Analytical Sciences, UK*

[4] *Manchester BIOGEL, 19F4 Mereside, Alderley Park, Alderley Edge, UK*

Corresponding author's email: a.saiani@manchester.ac.uk

The use of non-covalent self-assembly to construct materials has become a prominent strategy in biomaterials science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from cell culture and tissue engineering to in-vivo cell and drug delivery.[1] A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is de-novo designed peptides.[2] The β-sheet motif is of particular interest as short peptides can be designed to form β-sheet rich fibres that entangle and consequently form very stable hydrogels. These hydrogels can be easily functionalised using specific biological signals and can also be made responsive through the use of enzymatic catalysis [3-4] and/or conjugation with responsive polymers [5]. Through the fundamental understanding of the self-assembly and gelation of these peptides across length scales [6-8] we have been able to design hydrogels with tailored properties for a range of applications including for the culture of a variety of cells[9-11], injectable and sprayable hydrogels for cell and drug delivery [12-13] as well as shear thinning hydrogel for 3D bio-printing [14-15]. The intrinsic biocompatibility [16] and low immunogenicity [17] of these materials makes them also ideal for TERM applications. Recently we have demonstrated their potential in a range of TERM applications including, oesophagus [18], nerve [19], intervertebral disk [20] and cardiac [21] regeneration.

Keywords

Self-assembly; Peptide; Hydrogels

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Abstract **#765**

Mechanophysical and microbiological assessment of poly(epsilon-caprolactone)/poly (ethylene glycol) based electrospun nanofibrous mats loaded with an antibacterial for wound tissue engineering applications

Husam M Younes P[1], Sandi Ali Adib [1]

[1] *Office of Vice President for Research & Graduate Studies, Qatar University, Doha, Qatar.*

Corresponding author's email: husamy@qu.edu.qa

PURPOSE

The purpose of this study is to fabricate and characterize mechanically optimized biodegradable electrospun nanofibrous scaffolds (BENS) prepared using the hydrophilic Poly (ethylene glycol) (PEG35000) with the hydrophobic Polycaprolactone (PCL) co-polymers and assess the impact of loading them with Amoxicillin Trihydrate (AMX) on their mechanophysical and microbiological.1,2

EXPERIMENTAL METHODS

Blank and AMX-loaded BENS were fabricated using PEG35000 and PCL. Morphology of BENS was assessed using SEM. FT-IR was used to identify the interaction between PEG35000 and AMX. DSC was used to determine BENS crystallinity and thermal behavior. The XRD analysis was utilized to identify the changes in the BENS crystalline pattern. The prepared BENS were also subjected to mechanical testing and their in vitro antibacterial activity against common skin pathogens was also tested

RESULTS AND DISCUSSION

Various weight percentage ratios of PCL75%: PEG25%, PCL50%: PEG25%, PCL25%: PEG75% in chloroform were prepared. The DSC and FT-IR analyses showed that the polymers and AMX structures were not affected by the electrospinning. The PCL75%: PEG25% mix resulted in the optimal mechanical strength and used for preparing the AMX-loaded BENS. The DCS, FT-IR, and XRD studies confirmed the existence of AMX in the BENS and its dispersion and transformation into an amorphous state. The in vitro antibacterial assay confirmed the efficiency of the drug-loaded fibers against the common skin pathogens.

CONCLUSION

Our findings showed that PEG & PCL based BENS possessed enhanced mechanical and microbiological properties that made them promising candidates for wound healing applications.

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Abstract **#766**

LOCAL ACTIVITIES OF THE MEMBRANES ASSOCIATED WITH GLYCOSAMINOGLYCAN-CHITOSAN COMPLEXES IN BONE CELLS

Hsin-I Chang[P][1], Min-Fang Yang[1]

[1] *National Chiayi Univesity*

Corresponding author's email: hchang@mail.ncyu.edu.tw

Chitosan is a cationic polysaccharide derived from the partial deacetylation of chitin. Hyaluronic acid (HA), chondroitin sulfate (CS) and Heparin (HP) are anionic glycosaminoglycan (GCGs) which can regulate osteogenic activity. In this study, chitosan membranes were prepared by glutaraldehyde crosslinking reaction and then composited with three different types of GCGs. 7F2 osteoblast-like cells and macrophages Raw264.7 were studied in vitro for the effect of chitosan membranes on osteometabolism. Although chitosan membranes are highly hydrophobic, the membranes associated with GCG-chitosan complexes have showed about 60-80% cell attachment. Furthermore, the membranes associated with HP-chitosan complexes could increase ALP activity, up-regulate OPG/RANKL mRNA ratio and inhibit COX-2 mRNA expression in comparison with chitosan films only. All membranes could also inhibit MMP-3 mRNA expression. On the other side, three types of the membranes associated with GCG-chitosan complexes could significantly inhibit LPS induced-Nitric Oxide expression. In addition, chitosan membranes associated with HP and HA can down-regulate Tartrate-resistant acid phosphatase (TRAP) activity but not CS-chitosan complexes. Based on these results, we conclude that chitosan membranes associated with HP can increase ALP activity and up-regulate OPG/RANKL ratio in osteoblasts and chitosan membranes associated with HP and HA could reduce TRAP activity in osteoclasts.

Abstract **#767**

INNOVATIVE INJECTABLE AND POROUS HYDROGELS AS A SUPPORT FOR STRIATED SKELETAL MUSCLE TISSUE ENGINEERING

Louise Griveau[1,2], Emilie Christin[3], Heloise Le-Goff[1], Clémence Drouglazet[1], Baptiste Robbiani[1], Romain Debret[2], Vincent Gache[3], Jérôme Sohier[1]

[1] *MATEIS CNRS UMR 5510 - I2B team*

[2] *LBTI CNRS UMR 5305*

[3] *INMG CNRS UMR 5310 - INSERM U1217 - MNCA team*

Corresponding author's email: louise.griveau@gmail.com

VVolumetric muscle loss (VML) decreases muscle regeneration capacity and lacks treatments. Injectable hydrogels are promising therapeutic candidates but their potential strongly relies on the presence of porosity allowing cell infiltration and vascularization. Therefore, this study aimed (1) to create injectable and porous hydrogels based on poly-lysine dendrimers (DGL)/NHS-polyethylene-glycol (PEG) through an effervescent approach and (2) to evaluate its ability to sustain muscle cells progenitor differentiation for skeletal muscle regeneration.

We found that effervescent porous hydrogels (EPHs) of modular mechanical properties could be prepared by dissolving acetic acid and potassium carbonate to DGL and PEG solutions, to create a spontaneous, homogeneous and interconnected porosity, remnant of the stabilized CO2 bubbles entrapment in simultaneously cross-linked hydrogels. The use of sole precursor solutions allows to inject the formulations with a dual syringe and a static mixer, leading to porous hydrogels that were proven biocompatible by subcutaneous injection in mice.

As a striking result, primary human myoblasts[1] (phMs) seeded into 3D EPHs showed extensive myotube formation and visible striation after 6 days in serum-depleted medium with spontaneous contractions. Quiescent satellite cells presence inside EPHs was confirmed with myogenic regulatory factor Pax7 up to 14 days after differentiation, consolidating EPHs potential to support tissue formation by replenishing stem cell niches.

In conclusion, we describe a novel porous hydrogel with potential as scalable solution for VML treatments within a swift and straightforward injectable delivery, which provides an optimal support for muscle cells progenitors to differentiate into contractile myotubes while maintaining a pool of muscle stem cells.

Keywords

Porous and injectable hydrogels; Effervescent hydrogels; Skeletal muscle regeneration

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Abstract **#768**

MONOCYTE EXTRAVASATION IN AN ORGANOTYPIC MICROFLUIDIC MODEL OF OSTEOARTHRITIC JOINT

Silvia Lopa[P][1], Carlotta Mondadori[1], Silvia Palombella[1], Shima Salehi[1], Giuseppe Talò[1], Roberta Visone[2], Marco Rasponi[2], Alberto Redaelli[2], Valerio Sansone[3], Matteo Moretti[1,4]

[1] *IRCCS Istituto Ortopedico Galeazzi, Cell and Tissue Engineering Laboratory, Milan, Italy*

[2] *Department of Electronics, Information & Bioengineering, Politecnico di Milano, Milan, Italy*

[3] *IRCCS Istituto Ortopedico Galeazzi, Milan, Italy*

[4] *Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Lugano, Switzerland*

Corresponding author's email: silvia.lopa@grupposandonato.it

Introduction

Osteoarthritis (OA) is a multi-tissue disease in which the excessive infiltration and accumulation of monocytes in the synovium contributes to joint damage. Here, we created an organotypic microfluidic model recapitulating monocyte extravasation under inflamed OA-like conditions to investigate this dynamic process.

Methods

Synovium and cartilage were mimicked using hydrogel-embedded human OA synovial fibroblasts and chondrocytes. A perfusable endothelialized channel intervened in the synovium to mimic a post-capillary venule. Another channel was dedicated to synovial fluid injection. The endothelial monolayer was subjected to fluid flow and TNF-α before monocyte injection. Monocyte extravasation in response to a chemokine mix or OA synovial fluid was quantified and compared to a control group (culture medium).

Results

TNF-α and fluid flow synergistically induced the expression of ICAM-1 and VCAM-1 in endothelial cells. Validation experiments proved that monocyte extravasation was significantly higher in the presence of chemokines compared to the control group. Finally, we demonstrated that synovial fluid from OA patients induced a significantly higher number of monocytes to extravasate compared to the control group. Monocyte extravasation was enhanced by endothelium pre-activation.

Discussion & Conclusions

Our microfluidic OA joint model was successfully used to study monocyte extravasation in OA-like conditions, providing the first direct evidence that OA synovial fluid acts as a chemoattractant and induces monocytes to cross the endothelial barrier and invade the synovial compartment. We are currently assessing the effect of several molecules in modulating monocyte extravasation in obese and normal-weight OA patients.

Acknowledgments

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Keywords

Microfluidic; Monocyte; Extravasation

Abstract **#769**

IN VITRO DEVELOPMENT OF 3D CELL-DERIVED EXTRACELLULAR MATRICES FOR COLORECTAL CANCER MODELING

Gerard Rubi-Sans[1,2], Agata Nyga[3,1], Jordi Camps[4], Soledad Perez-Amodio[1,5], Miguel A Mateos-Timoneda[6,1], Elisabeth Engel[1,2,5], Bárbara Blanco-Fernández[P][7]

[1] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain*

[2] *CIBER en Bioingeniería, Biomateriales y Nanotecnología (CIBER-BBN), Madrid, Spain*

[3] *MRC Laboratory for Molecular Biology, Cambridge Biomedical Campus, Cambridge, UK*

[4] *Gastrointestinal and Pancreatic Oncology Group, Institut D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), CIBEREHD, Barcelona, Spain; Unitat de Biologia Cel·lular i Genètica Mèdica, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

[5] *Dept. Materials Science and Engineering, EEBE, Technical University of Catalonia (UPC), Barcelona, Spain*

[6] *Bioengineering Institute of Technology, Universitat Internacional de Catalunya (UIC), Barcelona, Spain*

[7] *Institute for Bioengineering of Catalonia (IBEC)*

Corresponding author's email: bblanco@ibecbarcelona.eu

KEYWORDS: Cell-derived matrix, Tumor modelThe use of cell-derived matrices (CDM) is a promising alternative to decellularized tissues/organs consisting of a complex assembly of macromolecules. 3D cell-cultured PLA microparticles combined with macromolecular crowding (MMC) effect, hold a great potential to develop bioactive materials for disease modeling applications. We propose CDMs as colorectal tumor models for personalized medicine by mimicking tissue microenvironment properties1.CDMs are produced by seeding human mesenchymal stem cells on fibronectin-coated PLA microparticles2 and cultured in presence of MMCs. Obtained CDMs are biochemically characterized by immunostaining and mass spectrometry; gene expression by qRT-PCR and RNA-sequencing; and mechanical properties by atomic force microscopy. Decellularized and particle-free CDMs are recellularized with colorectal cancer cells and cancer associated fibroblasts (CAFs)3 to further characterize cell-cell interactions, gene expression and CDM-remodeling potential. Obtained CDMs are compared with human colorectal tumor samples from patients.MMC effect enhances protein deposition in CDMs. Collagen types I, III and fibronectin are CDMs' main constituents, resembling colon tumor extracellular matrix. Decellularized and particle-free CDMs were recellularized with colon cancer cells and CAFs. Cancer CDMs' characterization is taking place to develop an in vitro tumor model to understand cancer promoting mechanisms, develop patient-specific drug screening platforms and to identify potential therapeutic targets.CDMs composition and the tunable matrix stiffness provides reproducible tissue microenvironment. By repopulating CDMs with cancer and stroma cells, we pretend to mimic native tissue structure and properties to obtain a promising platform for in vitro tumor model generation.Authors thank MINECO (MAT2015-68906-R) and RTI2018-096320-B-C21 for funding

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Abstract **#770**

COLONIZATION OF THE ORGAN-SPECIFIC MATRICES BY TUMOR CELLS: THE ROLE OF EXTRACELLULAR MATRIX COMPARTMENTALIZATION AND IMPLICATIONS FOR CANCER TREATMENT AND DIAGNOSTICS

Anna Guller[P][1,2,3], Vlada Rozova[2,4,5], Annemarie Nadort[2,4], Sameera Iqbal[2,4], Mina Ghanimi Fard[2,4], Lindsay Parker[2,4], Shivani Sachdev[4], Dmitry Polikarpov[2,4], Mahsa Vaez Zadeh[2,4], Aleksandra Polikarpova[4,1], Zahra Khabir[2,4], Nicki Packer[2,4], Itsu (Yi) Sen (Qian)[4], Andrei Zvyagin[2,4,3], Ewa Goldys[2,1]

[1] *University of New South Wales, NSW, 2032, Australia*

[2] *ARC Centre of Excellence for Nanoscale BioPhotonics, Australia*

[3] *Sechenov University, Moscow, 119992, Russia*

[4] *Macquarie University, NSW 2109, Australia*

[5] *University of Melbourne, Australia*

Corresponding author's email: a.guller@unsw.edu.au

Metastatic colonization of the organs is the main cause of cancer-related deaths. The patterns of cancer cells dissemination of the in different organs are not completely explainable by the blood circulation and vascularization, implying that local and organ-specific factors may contribute to the observed metastatic phenomena. In this work, we applied tissue engineering methodology to explore the effect of organ-specific extracellular matrix (OS-ECM) on cancer colonization rates and strategies.

Twelve types of OS-ECM scaffolds with preserved composition and histoarchitecture were prepared by mild decellularization, seeded with various types of cancer and cultured in vitro. The model tumors were assessed by pathology methods, digital image analysis, and machine learning approaches. In a separate experiment we seeded breast cancer cells on collagen I-coated silicone surfaces with controlled stiffness (from 0.2 to 64 kPa) and examined stiffness-induced phenotypical changes to correlate with our 3D culture observations.

Most OS-ECMs contained two compartments, originating from the organ parenchyma and stroma, respectively. They had different roughness/stiffness but similar chemical composition. Cancer cells revealed alternative attachment, migration and morphology in these OS-ECM compartments during the first 3 weeks in vitro, followed by spongification and total colonization of the matrix. The breast cancer cells phenotypic plasticity was upregulated across various stiffness levels. OS-ECM also significantly decreased sensitivity of cancer cells to chemotherapy.

This work demonstrated that virgin OS-ECM provides a dual morphogenetic niche which induces alternative cellular behaviour and enhancing adaptiveness of cancer cells. The implications of our findings for cancer diagnosis and treatment will be discussed.

Abstract **#771**

YAP MECHANOPARACRINE EFFECT CONNECTS NUCLEOSKELETON INTEGRITY WITH EXTRACELLULAR MATRIX REMODELING

Jorge Oliver-De La Cruz[P][1], Ana Rubina Perestrelo[1], Fabiana Martino[1], Jan Vrbský[1], Antonio Pompeiano[1], Helena Duríková[1], Guido Caluori[2], Martin Barrios-Llenera[1], Stefania Pagliari[1], Giancarlo Forte[1]

[1] International Clinical Research Center (FNUSA-ICRC), St. Anne's University Hospital, Brno, Czech Republic

[2] Unit for Lymphopoiesis, Immunology Department, INSERM U668, University Paris Diderot, SorbonneParis Cité, Cellule Pasteur, Institut Pasteur, Paris, France

Corresponding author's email: oliver.jorge@fnusa.cz

YAP is a pro-fibrotic co-transcriptional activator which promotes extracellular matrix (ECM) deposition. While it is now credited of acting in a positive loop controlling cytoskeleton tension (1), the interplay between ECM and cytoskeleton through YAP is not completely clear. By combining decellularized matrices (dECM), RNA sequencing and immunostainings, with CRISPR/overexpression techniques, we defined YAP contributes to ECM generation in different cell types. We described how YAP controls the expression of individual matrisome components, and the deposition, remodeling and degradation of surrounding ECM. YAP mutant cells presented reduced cellular stiffness, that was mirrored by a deranged nucleoskeleton and decreased nuclear tension, inducing morphological abnormalities and alterations in the levels and distributions of several components of the nuclear envelope such as Nesprins and Lamins. Cell stiffness and nucleoskeletal organization were partially restored by growing cells onto dECM produced by cells expressing pathologically high YAP levels, with a significant recovery in Lamin A/C levels and nucleoplasmic retention of its phosphorylated forms. This rescue was independent of actomyosin tension and microtubular structures. In conclusion, we describe a mechanoparacrine effect of YAP by which cells, through the control of ECM production, can affect the cellular and nucleoskeletal mechanics of neighbor cells, independently of their own YAP intracellular activity. Our results suggest that combined therapies targeting simultaneously YAP and ECM could be potentially more beneficial in cancer therapeutic approaches.

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Abstract **#772**

IN VIVO IMMUNOBIOLOGICAL PROFILING OF BLENDED ALOE VERA NANOMESHES BOOSTED WITH ENDOMETRIAL MESENCHYMAL STEM CELLS FOR ADVANCING WOMEN'S HEALTH

Shayanti Mukherjee$_P$[1,2], Saeedeh Darzi[1], Shanilka Leitan[1,2], Jerome Werkmeister[1,2], Caroline Gargett[1,2]

[1] *The Ritchie Centre, Hudson Institute of Medical Research, Clayton VIC Australia 3168*

[2] *Department of Obstetrics and Gynaecology, Monash University, Clayton VIC Australia 3168*

Corresponding author's email: shayanti.mukherjee@hudson.org.au

Pelvic organ Prolapse (POP) is a condition effecting 1 in 4 women caused by injury and subsequent weakening of the vaginal wall during childbirth. Until recently reconstructive surgery augmented by synthetic non-degradable meshes were commonly used, however these meshes have been now banned owing to complications arising from severe foreign body responses (FBR). To promote tissue regeneration and mesh integration, we have developed nanostructured degradable meshes using electrospinning of polymer poly (L-lactic acid)-co-poly (ε-caprolactone) blended with Aloe Vera (PAV) boosted with endometrial mesenchymal stem cells (eMSCs). PAV meshes with and without eMSCs were implanted into a mouse model of POP and evaluated for mesh integration, fate and FBR response after 1 and 6 weeks. Our results show that meshes with eMSCs reduced cell adhesion and capsule formation at the mesh-host interface by 6 weeks compared to PAV alone. Scanning electron microscopy (SEM) revealed formation of neo blood vessels, ECM as well as improved mesh integration at the mesh interface. We are currently investigating the macrophage mediated FBR response with focus on M1 and M2 polarization. Our results highlight the potential of such meshes as an alternative POP treatment that may address a major unmet women's health need.

Abstract **#774**

DESIGN, TOOLS AND BIOMATERIALS FOR SYNTHETIC MORPHOGENESIS

Aurelien Forget$_P$[1]

[1] *Institute for Macromolecular Chemistry*

Corresponding author's email: aurelien.forget@makro.uni-freiburg.de

Answering the question of how organs develop has been driven by focusing on the cell's gene expression. However, during the development of organs, materials surrounding the cells play a key role in guiding their organizations by presenting information in the form of gradients. By adapting novel design strategies and manufacturing tools with biomaterials, we now have the capacity to reproduce graded architectures needed to achieve synthetic morphogenesis.

Endeavours to create synthetic organs have been focusing on reproducing the cellular organization of mature tissue using diverse biofabrication techniques. Away from the technical race aiming to achieve faster and more precise cell deposition to reproduce the complex cell organization observed in adult tissue, we would like to present an approach where a synthetic environment would be programmed to direct the organization into a mature organ following the design rules of organ development.

In this talk conceived in three parts, we will first present our most recent work on how we can apply architectural tools to adopt design theory based on developmental principles.1 We will then proceed to showcase our manufacturing advances for the creation of graded objects mimicking developmental gradients. Finally, we will present our clickable biomaterials that can be functionalized with peptides during 3D printing, allowing us to create 3D patterns of biological information.

We envision these technological advances as a cornerstone to create multiple morphospaces where the principle of organ development can be tested in vitro to shape the future of regenerative medicine.

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Abstract **#775**

3D-BIOPRINTING SYSTEM FOR ENGINEERING COMPLEX, SOFT HYDROGEL SCAFFOLDS

Núria Torras Andrés[P][1], Jon Zabalo[1,2], Eduardo Abril[1], Elena Martínez[1,3,4]

[1] *Biomimetic Systems for Cell Engineering, Institute for Bioengineering of Catalonia, The Barcelona Institute of Science and Technology, Barcelona, Spain*

[2] *CEIT, Donostia-San Sebastian, Spain*

[3] *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina, Madrid, Spain*

[4] *Department of Electronics and Biomedical Engineering, University of Barcelona, Barcelona, Spain*

Corresponding author's email: ntorras@ibecbarcelona.eu

There is an increasing evidence that the tissue architecture has a strong influence on cell behavior and it is key to obtain functional tissue replicates (1). Hence, future improvements in the tissue engineering field will encompass tailoring three-dimensional (3D) cell microenvironments with the precision required to mimic in vivo features.

3D printing techniques have been positioned as a feasible alternative to conventional manufacturing techniques to achieve proper spatial resolution in a high-throughput manner, mainly due to high adaptability and reduced costs, becoming one of the current hot topics in applied research (2,3). However, requirements in terms of resolution, mechanical properties, or biocompatibility to obtain biomimetic 3D tissues demand further innovative approaches.

Here we present a fast, low cost and versatile printing method based on visible light polymerization for the fabrication of 3D bioengineered substrates and cell-friendly interfaces using photopolymerizable hydrogel-based bioinks. These bioinks are highly transparent and have low molecular content, mimicking the soft mechanical properties of the tissues.

With the appropriate combination of bioink composition, accurate selection of the printing parameters, and optimized designs we succeeded with prints for different applications, including cell culture substrates, microfluidic channels and tissue constructs for in vitro assays, compatible with the standard cell culture techniques. As a proof-of-concept, we developed functional scaffolds mimicking the 3D microstructure of the small intestine, containing both the epithelial and stromal compartments, using cell-laden bioinks.

Through this versatile top-down technique we demonstrated the potential of the light-based 3D bioprinting technology, contributing on providing alternatives beyond tissue engineering state-of-the-art.

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Abstract **#776**

HYALURONIC ACID/CALCIUM PHOSPHATE/STRONTIUM RANELATE COMPOSITE HYDROGELS FOR CONTROLLED DRUG DELIVERY

Dagnija Loca$_P$[1,2], Alise Svarca[2]

[1] *Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia*

[2] *Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Pulka 3, Riga, LV-1007, Latvia*

Corresponding author's email: dagnija.loca@rtu.lv

INTRODUICTION: Fragility of osteoporotic bone is challenging, while the patients in most cases also possess the age-related disorders, highly influencing the fracture healing around the surgical sites [1]. Strontium ranelate (SrRan) has been found as an effective drug able to increase and stabilize the bone mineral density. Hyaluronic acid (HA) and calcium phosphates (CaP) have attracted an interest due to their biocompatibility and osteoconductivity, respectively. Most frequently HA based hydrogels consist of randomly interconnected polymer chains and drugs when incorporated in such structures without any covalent linkage to the HA network are released rapidly not serving as a prolonged release systems [2]. The aim of the current study was to evaluate the potential of HA/CaP/SrRan composite hydrogels as prolonged release drug delivery vehicles.

METHODS: HA/CaP/SrRan composite hydrogels were pepared mixing all components with 0.25M NaOH and BDDE. Prepared systems were crosslinked for 24 h at 45 °C, neutralized in 0.9% NaCl solution and used for the determination of SrRan release via UV-VIS.

RESULTS: All prepared hydrogels were characterized with active substance burst release (>30%) within the first 24 h, followed by up to 77% of SrRan release in dissolution media within period of 75 days.

DISCUSSION & CONCLUSIONS: During the research it was established that SrRan release from prepared compositions strongly depends on the route of CaP nanoparticle introduction into the hydrogel systems as well as on interactions between HA and drug molecules.

ACKNOWLEDGEMENTS: Authors acknowledge financial support from H2020 research and innovation programme under the grant agreement No 857287.

Keywords

hydrogels; drug delivery; bone regeneration

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Abstract **#777**

SMART WRINKLE TOPOGRAPHY FOR DICTATING CELL ACTIVITIES

Ryan Dimmock$_P$[1], Yibin Fu[2], Ying Yang[1]

[1] *School of Pharmacy and Bioengineering, Keele University, Stoke-on-Trent ST4 7QB, UK*

[2] *School of Computing and Mathematics, Keele University, Staffs ST5 5BH, UK*

Corresponding author's email: r.l.dimmock@keele.ac.uk

Control of cellular behaviour using external stimuli is a concept widely adopted in many biomedical applications, including regenerative medicine. Within these applications there is either a desire to control differentiation or preserve a particular phenotype, including stem cell culture and its applications. Material surface manipulation facilitates the replication of tissue anatomic structures through the creation of wrinkles across biomaterial surfaces (1). The underlying principle governing wrinkle formation is that the surface stiffness must be greater than the underlying bulk, when this stiffness is great enough the surface wrinkles upon physical deformation of the body. Therefore creation of polymer laminated elastomer blocks and O2 plasma treatment of elastomer are feasible techniques to achieve controllable and tuneable wrinkles (1). Previous work in the regenerative medicine field has featured static substrates for cellular topography replication, however a dynamic topography system is required as well. In this study, multiple parameters have been optimised to generate wrinkles with defined frequency, width and depth. To facilitate the generation of tuneable surface wrinkling, spatially and temporally, the laminated block method was combined with a small-scale bioreactor system fitting within a conventional cell culture vessel was developed. Using the laminated block system, a dynamic returnable wrinkled topography was achieved. The wrinkled surfaces generated by both methods have demonstrated the ability to align/ localise cells in discrete regions and alter their morphology. From these developments and utilising further optimisations, these methods will be useful studying cell response to the dynamically tuneable topography, including mimicking epithelial degenerative diseases.

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Abstract **#780**

THE EFFECT OF PORE SIZE ON OSTEOGENIC DIFFERENTIATION OF BONE MARROW MESENCHYMAL STEM CELLS IN SILK FIBROIN SCAFFOLDS

Chengchong Ai[1,2], James Goh[1,2]

[1] *NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore*

[2] *Department of Biomedical Engineering, National University of Singapore*

Corresponding author's email: e0360527@u.nus.edu

Porous silk fibroin (SF) scaffolds have been widely used for bone tissue engineering (BTE), and the pore structure of scaffolds plays an important role in osteogenesis. The pore size of SF scaffolds reported in literature for BTE is quite varied, ranging from 60 to 700 µm[1, 2]. This study aimed to investigate the effect of pore size on osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) in vitro. HFIP-derived SF scaffolds with pore diameters in the ranges of 355-400, 250-315, 160-220 µm were prepared by salt leaching methods, named as Large, Medium, and Small group. Porcine BMSCs were seeded on scaffolds and cultured in osteogenic medium for 21 days to evaluate cell proliferation, alkaline phosphatase (ALP) activity, calcium deposition, gene expression of osteogenic markers, and histological performance. Cell metabolic rate in each group increased significantly with time, but there is no statistical difference on the reduction rate at each time point among three groups. On day 21, ALP/DNA and calcium/DNA in Small group was significantly higher than that in the large group. Col I mRNA expression on day 7, OPN mRNA expression level on day 14, and OCN mRNA expression level on day 21 in Small group is higher. Immunohistochemical staining results on day 21 showed that Col I and OCN in Small group were more highly expressed. In conclusion, the HFIP-derived SF scaffolds with a mean pore diameter of 0.19 µm was optimal for osteogenic differentiation of BMSC in vitro.

Keywords

Silk fibroin; pore size

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Abstract **#781**

ASSESSING THE EFFECTS OF ARTICULAR JOINT INFLAMMATION IN A MICROFLUIDIC VASCULARIZED OSTEOCHONDRAL MODEL

Matteo Moretti[P][1,2], Shima Salehi[1], Giovanni Stefano Ugolini[3], Marco Rasponi[3], Silvia Lopa[1]

[1] *IRCCS Istituto Ortopedico Galeazzi, Cell and Tissue Engineering Laboratory, Milan, Italy*

[2] *Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Lugano, Switzerland*

[3] *Department of Electronics, Information & Bioengineering, Politecnico di Milano, Milan, Italy*

Corresponding author's email: silvia.lopa@grupposandonato.it

Introduction

Osteoarthritis (OA) is characterized by cartilage degradation and alterations in subchondral bone remodeling and angiogenesis. Here, we exploited a microfluidic device to model the osteochondral interface for investigating the expression of factors involved in these processes in healthy and OA-like conditions.

Methods

The cartilage compartment was modeled by fibrin-embedded human articular chondrocytes. The cellular components of the bone part namely osteoblasts, osteoclasts, and endothelial cells were embedded in fibrin hydrogel enriched with calcium phosphate nanoparticles [1]. To induce OA-like conditions, the experimental group was treated with IL-1β. The expression of markers related to cartilage degradation, bone remodeling, and angiogenesis was assessed.

Results

Matrix metalloproteinases expression by osteoclasts was evident in both groups, being slightly higher in IL-1β treated samples. Differently, chondrocytes expressed matrix metalloproteinases only when treated with IL-1β. The expression of RANK as a factor regulating osteoclastogenesis was higher in the IL-1β treated group. Regarding angiogenesis, vascular endothelial growth factor was highly expressed only by endothelial cells in control samples, whereas its expression by osteoclasts was strongly upregulated when the samples were treated with IL-1β.

Discussion & Conclusions

We developed the first microfluidic vascularized osteochondral model in which cartilage and bone compartments are in direct contact. We demonstrated that inflammatory conditions stimulate the expression of matrix degradation factors, regulate bone remodeling, and involve osteoclasts in angiogenesis augmentation. We are currently analyzing the response of the model to an anti-inflammatory drug, Dexamethasone, in terms of all the aforementioned factors.

Acknowledgments

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Abstract **#782**

DEVELOPMENT OF AN IN VITRO COCULTURE MODEL CONNECTING BILE CANALICULI AND BILE DUCTS AND ITS MINIATURIZATION

Tsubasa Kaneko[P][1], Ryota Kaku[1], Tadahiro Yamashita[1], Ryo Sudo[1]
[1] *Department of System Design Engineering, Keio University*
Corresponding author's email: tsubasakaneko@keio.jp

Bile secreted from hepatocytes has function to assist digestion and absorption of lipids. However, excessive accumulation of bile acids in hepatocytes causes necrosis. Therefore, bile must be transported from bile canaliculi, which are formed by hepatocytes, to bile ducts, which are formed by cholangiocytes. From the viewpoint of liver tissue engineering, it is important to construct bile canaliculi and bile ducts in vitro. Although previous studies reported construction of each tissue, little is known about a coculture in which bile canaliculi and bile ducts are connected. Here, we induced connection of bile canaliculi and bile ducts by the coculture of rat primary small hepatocytes and cholangiocytes. Specifically, we cultured cholangiocytes on collagen gel for 3 days, then started coculture by adding small hepatocytes. The cells were then overlaid with collagen gel and dimethylsulfoxide was added to culture medium for inducing bile duct formation. We confirmed that small hepatocytes and cholangiocytes jointly formed a luminal structure at the junction of bile canaliculi and bile ducts. We also investigated the CYP activity of small hepatocytes in the coculture. The results showed that the CYP activity of small hepatocytes in the coculture was comparable to that in the single culture. Finally, this coculture model was reproduced in a small culture device for miniaturizing the coculture model and for easy analysis of the connection of bile canaliculi and bile ducts. We fabricated a small PDMS culture device with two compartments, in which bile canaliculi or bile ducts were constructed.

Keywords
bile duct; bile canaliculi

Abstract **#783**

HEMOCOMPATIBILITY IMPROVEMENT OF A DECELLULARIZED LIVER SCAFFOLD THROUGH CONSTRUCTION OF VASCULAR NETWORKS INCLUDING SINUSOID-SCALE MICROVESSELS

Masayuki Shinohara[P][1], Masafumi Watanabe[1], Tadahiro Yamashita[1], Ryo Sudo[1]

[1] *Department of System Design Engineering, Keio University*

Corresponding author's email: masayuki.s.205@keio.jp

There is a growing demand for transplantable bioengineered livers for patients with end-stage liver diseases due to a shortage of donor livers. Decellularized liver scaffolds are thought as a promising platform for constructing bioengineered livers because of their intact scaffold architecture, ECM composition, practical scale, and low immunogenicity. Although bioartificial liver surrogates have been developed using decellularization techniques, hemocompatibility of vascular networks in the liver surrogates is not enough, which is the main cause of the difficulty in long-term transplantation. Therefore, hemocompatibility improvement of liver surrogates is important, which can be achieved by re-endothelialization of vascular lumens. Here, we hypothesized that construction of sinusoid-scale microvessels in a decellularized liver could provide better hemocompatibility of liver surrogates. We recently established a method to construct vascular networks including sinusoid-scale microvessels in a decellularized liver1. In the present study, we tested hemocompatibility of the constructed vascular networks in a decellularized liver. Specifically, a re-endothelialized liver on day 2 was perfused with diluted rat blood. The number of platelets attached to the scaffold was counted to evaluate hemocompatibility of the re-endothelialized livers. The number of platelets in a decellularized liver with sinusoid-scale microvessels was five times smaller than that without sinusoid-scale microvessels and about ten times smaller than that in an acellular liver scaffold. These results could be attributed to both physical effect of covering vascular lumens with endothelial cells and endothelial biochemical antithrombic effect.

Keywords

decellularized liver; re-endothelialization; hemocompatibility

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Abstract **#784**

A SIMPLE AND SCALABLE TECHNOLOGY FOR MICRO AND NANO-TOPOGRAPHIC PATTERNING OF STANDARD CELL CULTUREWARE TO SCREEN CELL BEHAVIOUR IN VITRO

Victor J. Cadarso[P][1], Hazem Abdelmaksoud[1,2], James Carthew[1], Karla Cowley[3], Kaylene Simpson[3], Joachim Spatz[4], Juergen Brugger[5], Helmut Thiessen[6], Roey Elnathan[1], Nicholas Voelcker[1,2,6], Jessica Frith[1], Victor J Cadarso[1]

[1] *Monash University*

[2] *Melbourne Centre for Nanofabrication*

[3] *Victorian Centre for Functional Genomics*

[4] *Max Planck Institute for Medical Research*

[5] *EPFL*

[6] *CSIRO*

Corresponding author's email: victor.cadarso@monash.edu

Substrate topography at the micro and nanoscale is emerging as a powerful tool to manipulate cell behaviour, but studies into these effects are hampered by a lack of access to topographies that can be readily incorporated with the standard biological techniques and methods of analysis. Current techniques to produce micro and nanopatterned substrates are often difficult to produce across a surface area large enough to perform biological analyses, require time consuming and tricky manipulation of substrates into standard cell culture plates and are typically not made of the same material (polystyrene), which further complicates any comparison with typical cell culture datasets.

We have developed a simple and scalable technology to produce precise micro- and nano-scale topographic patterns on the surface of standard cell cultureware. We show that the characteristics of the surface are maintained and that the process does not impede imaging. We further highlight the utility of this technology by performing a high-throughput screen to interrogate the effects of 12 surface topographies across 5 different cell types and are able to identify common and distinct cellular response to topographic features as well as image-based signatures to identify specific cell phenotypes. This technology could open up the potential of micro/nano-topographies in the culture and screening across an enormous range of biological applications.

Abstract **#785**

A SYNTHETIC BIOMATERIALS APPROACH TO IMPROVE ISLET TRANSPLANTATION

Adam L. Stell$_P$[1], Sami G. Mohommed[2], Rick de Vries[2], Marten A Engelse[3], Elco de Koning[3], Vanessa L.S. LaPointe[2], Mireille M.J.P.E. Sthijns[2], Aart A. Van Apeldoorn[2]

[1] *MERLIN Institute, Maastricht University*

[2] *MERLN Institute, Maastricht University*

[3] *Leiden University Medical Center*

Corresponding author's email: a.stell@maastrichtuniversity.nl

As a treatment for type I diabetes, clinical islet transplantation in which donor islets of Langerhans are transplanted in the liver of the recipient has become a viable option for patients. However, the success of this procedure is limited by factors including ischemia, host immunological factors, and delayed vascularization of the hypoxia-sensitive islets. One solution would be to use a synthetic biomaterial scaffold as a carrier for the transplanted islets, as it would allow for their transplantation into a more favorable normoxic environment and protect the cells from host immune reactions. To realize this potential solution, it is important that the synthetic biomaterial implant not interfere with the functionality and survival of the islets. In order to determine which biomaterials best meet this essential condition, we examined the interactions of human islets from six donors cultured on four clinically-approved materials: polyetheretherketone (PEEK), polyvinylidene fluoride (PVDF), polyphenylsulfone (PPSU) and polysulfone (PSU). Human islet morphology, viability, insulin secretion, functionality and gene expression were investigated to assess the suitability of these biomaterials as a carrier for transplanted islets. We found three of the synthetic biomaterials (PEEK, PPSU and PVDF) showed promise based on their overall favorable assay results, while the functionality of islets cultured on PSU resulted in significantly reduced insulin secretion from five of six donors. Our findings demonstrate that certain synthetic biomaterials can influence islet function and an examination of any synthetic biomaterial on islet function would be beneficial before selecting that material for fabricating an islet implant device.

Keywords

Biomaterial; islet encapsulation; type I diabetes

Abstract **#787**

DE NOVO BONE TISSUE REGENERATION USING BIOACTIVE SCAFFOLD PRESENTING NATIVE BONE MIMICKING BIOCHEMICAL AND BIOPHYSICAL FEATURES

Farhad Soheilmoghaddam[P][1], Justin Cooper-White[1,2]

[1] *Tissue Engineering and Microfluidics Laboratory, Australian Institute for Bioengineering and Nanotechnology The University of Queensland, Brisbane QLD 4072, Australia*

[2] *School of Chemical Engineering, University Of Queensland, St Lucia, QLD, Australia*

Corresponding author's email: m.soheilmoghaddam@uq.edu.au

A major limitation to the integration and survival of large tissue engineered bone at the site of implantation is the lack of an intrinsic vascular network. In this work, a customized cell patterning approach was developed to create a vascularized bone construct, made from a bilayered scaffold of electrospun PLGA/nHA composite fibres interleaved with a PEG based-hydrogel displaying an osteogenic peptide (OP), patterned with human bone marrow-derived mesenchymal stem cells (hBMSCs) and human endothelial cells (hECs). Utilizing this novel co-culture approach, a bone osteon mimic, comprised of lumen structure seeded with hECs surrounded by multilayers of hBMSC-laden PLGA matrix, was produced. The co-cultured PLGA-nHA-PEG-OP implant produced significant improvements in bone formation and cell maturation (even in growth/maintenance only medium) compared to hBMSC-based monocultured implants, confirmed through gene expression and protein secretion. Importantly, the co-cultured scaffolds showed promising results in terms of maintaining hEC morphology and function, even after 21 days, with VE-cadherin staining confirming the formation of tube-like structures between regions populated with hBMSCs and hECs. Osteocyte-like cells with multi-dendritic morphology, communicating with neighboring osteocyte-like cells, were observed throughout the scaffold. These osteocyte-like cells expressed early osteocyte marker gene, including E11/gp38 (E11) as well as late marker expression, including dentin matrix acidic phosphoprotein 1 (DMP1). The outcomes of this study demonstrate that when seeded with cell types constituent of the bone marrow microenvironment, these novel multilayered composite scaffolds, in presenting native bone-mimicking biochemical and biophysical features, are able to create osteocytes within vascularised osteon-like structures, in vitro.

Abstract **#788**

AN IN SILICO MODEL TO INFORM CELL TRANSPLANTATION USING BIOMATERIALS FOR PARKINSON'S DISEASE

Despoina Eleftheriadou[1,2,3], Victoria H. Roberton[1,2], Joanne Jones[4], Sarah Howlett[4], Venkat Pisupati[4], Rebecca J. Shipley[1,3], James B. Phillips[1,2], Simão Laranjeira[1,3]

[1] *UCL Centre for Nerve Engineering, London, UK.*

[2] *Department of Pharmacology, UCL School of Pharmacy, London, UK.*

[3] *UCL Department of Mechanical Engineering, London, UK.*

[4] *Department of Clinical, Neuroscience, Cambridge University, Cambridge, UK.*

Corresponding author's email: s.laranjeira@ucl.ac.uk

Parkinson's disease (PD) is a highly debilitating disorder of the nervous system that affects more than 1% of the population over the age of 60. Cell therapy has emerged as a promising strategy for modifying PD's progression [1]. However, early evidence suggests that a high percentage of implanted cells die, possibly due to mechanical stress during transplantation and the subsequent host immune and inflammatory responses. Encapsulating cells within a biomaterial could potentially confer protection and promote engraftment [2]. In this work, we propose a multidisciplinary approach to identify a suitable biomaterial and define the optimal seeding density, oxygen availability and spatial distribution of ventral midbrain dopaminergic progenitors differentiated from human pluripotent stem cells.

Initial experiments compared different biomaterials to identify those which protected the cells from mechanical stress during injection, with a blend of collagen and hyaluronic acid identified to be most appropriate. Additionally, the effects of cell seeding density and oxygen availability on cell survival were explored, revealing that these cells were able to tolerate hypoxic conditions associated with relatively high seeding densities and low levels of environmental oxygen in vitro.

Using an in-house mathematical framework, parameterized with the data acquired, the interactions between cell seeding density and relevant spatial and temporal distributions can be modelled and optimised in silico prior to in vivo testing [3]. This interdisciplinary approach combines experimental work with mathematical modelling to accelerate the clinical translation of new therapeutic strategies.

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Abstract **#789**

TRILAYERED ALIGNED ELECTROSPUN CONDUITS WITH ENCAPSULATED GDNF AND TACROLIMUS FOR AXONAL GUIDANCE AND REGENERATION IN PERIPHERAL NERVE REPAIR

Holly Gregory[P][1], James B Phillips[1], Victoria H Roberton[1], Stephen Hilton[1], Gareth Williams[1]
[1] *UCL School of Pharmacy*
Corresponding author's email: holly.gregory.18@ucl.ac.uk

The efficacious repair of severe peripheral nerve injuries is currently an unmet clinical need. These injuries require surgical intervention and the gold standard of repair, transplanting nerve tissue from elsewhere in the body to bridge the gap, suffers from donor-site morbidity, limited supply and graft diameter mismatch. Polymeric electrospun nanofibrous nerve conduits potentially offer an artificial alternative to this technique by providing guidance for the regenerating nerve, in order to achieve target reinnervation and functional recovery. Generating these conduits with aligned fibres and encapsulated drugs, alongside the post-fabrication seeding of therapeutic cells, may further improve regenerative outcomes by mimicking the native nerve structure and cellular environment.

Here, two conduits were developed with a trilayered wall material formed from randomly-orientated drug-eluting fibre mats sandwiched between two aligned polymer-only fibre layers. The central drug-eluting layer contained either glial cell line-derived neurotrophic factor (GDNF), a potent neurotrophic factor, or tacrolimus, an immunosuppressant known to promote nerve regeneration. The encapsulation of GDNF into polycaprolactone (PCL) fibres was achieved using emulsion electrospinning, and GDNF-PCL fibres were optimised for uniform morphology and sustained release of bioactive protein. Coaxial electrospinning was used to fabricate PCL fibres containing tacrolimus and also yielded fibres with consistent morphology and controlled drug release. The ability of the axon-interfacing layer of the wall material, aligned PCL-only fibres, to guide neurons was also explored. These conduits show promise for nerve repair and may support regeneration across gaps in nerve tissue as an alternative to the current gold standard.

Keywords
Electrospinning; Growth factors; Tacrolimus

Abstract **#790**

DUAL-FUNCTIONALIZED VISIBLE-LIGHT RESPONSIVE GELATIN BIOINK AS CARTILAGE BINDING GLUE AND MATRIX FOR 3D CHONDRAL REGENERATION

Florencia Abinzano[P1], Khoon Lim[2], Paulina Núñez Bernal[1], Pau Atienza Roca[2], Ane Albillos Sanchez[1], Iris Otto[1], Michiya Matsusaki[3], Tim Woodfield[2], Jos Malda[1,4], Riccardo Levato[1,4]

[1] *University Medical Center Utrecht, Department of Orthopedics, Utrecht, Netherlands*

[2] *CReaTE Group, University of Otago Christchurch, Christchurch, New Zealand*

[3] *Department of Applied Chemistry, Graduate School of Engineering Osaka University, Osaka, Japan*

[4] *Utrecht University, Department of Clinical Sciences, Utrecht, Netherlands*

Corresponding author's email: f.abinzano@umcutrecht.nl

Hydrogel bioprinting holds potential for capturing the zonal architecture of native cartilage, enhancing tissue regeneration[1]. Yet, fixation of hydrogel constructs is a major challenge due to limited lateral integration and the risks of damage to the surrounding tissue upon crosslinking. The aim of this study was to develop a printable hydrogel with potential for in situ cartilage repair, based on a cell-laden gelatin methacryloyl hydrogel modified with tyramine moieties (gelMA-Tyr) bearing dual crosslinking capacity.

Photo-induced gelation of the reactive acryl- and tyramine groups was triggered in one step via visible-light irradiation using Ru/SPS. GelMA-Tyr and gelMA were loaded with articular cartilage progenitor cells and cultured in vitro. Both gelMA and gelMA-Tyr supported cell survival and chondrogenesis in terms of viability, glycosaminoglycans production and compressive modulus.

GelMA-Tyr exhibited shear-thinning behaviour and could be printed using an extrusion-based platform, while showing shape retention post-printing[2]. The tissue-binding capacity was studied using a push-out test upon casting in chondral explants. The visible-light tyramine-methacryloyl dual crosslinking showed 15-fold the adhesive strength to native cartilage compared to Irgacure-gelMA.

Moreover, the Ru/SPS visible-light crosslinking system resulted on average on 57% higher cell viability of surrounding native cartilage when compared to UV-Irgacure crosslinking. Ru/SPS crosslinkers did not show oxygen inhibition, facilitating the use of the gel in situ.

Overall, visible-light crosslinkable gelMA-Tyr hydrogels in combination with the dual crosslinking mechanism triggered by Ru/SPS demonstrated potential for direct delivery and integration into damaged cartilage. Its potential as a bioink enhances the possibilities for the repair of complex, patient-specific defects.

Keywords

Cartilage repair; Biofabrication; Bioglue

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Abstract **#791**

IN SILICO MODELLING FRAMEWORK TO INFORM GUIDANCE STRATEGIES FOR NEURITE POPULATIONS POST PERIPHERAL NERVE INJURY

Simão Laranjeira[P][1,2], Victoria H. Roberton[3,2], James B. Phillips[3,2], Rebecca J. Shipley[1,2]

[1] *UCL Mechanical Engineering, London, UK.*

[2] *UCL Centre for Nerve Engineering, UK.*

[3] *Department of Pharmacology, UCL School of Pharmacy, London, UK.*

Corresponding author's email: s.laranjeira@ucl.ac.uk

Peripheral nerve injury (PNI) affects 1M people in Europe and the USA p.a., and patients often experience debilitating symptoms [1] including loss of sensation and paralysis. The associated healthcare and societal costs amount to around 100 billion $/year in the USA alone [2].

The current gold-standard for large gaps injuries consists of connecting the two stumps through microsurgical autografts. However, this requires the extraction of healthy nerve from patients, resulting in donor site morbidity and often poor functional outcomes [2]. Researchers aim at replacing grafts by developing nerve repair conduits (NRC) that provide the required durotactic and chemotactic cues to promote neurite regeneration, however, none yet have surpassed the graft [3]. Here, an in silico model is integrated into the NRC design workflow, that allows for more sophisticated considerations of the distribution of cues within the device. Specifically, we focus on including both motor and sensory neurite populations, which have marked differences in terms of their growth rates and response to cues, and this could contribute significantly to the limitations of the current designs [4,5].

A discrete-continuous modelling framework was devised to consider the two neurite populations, where they differ by their response to cues and their growth rate. The model is parameterised using in vivo data on neurite counts at the proximal and distal stumps. It is then used to propose the optimal organisation of chemical and durotactic cues within novel NRC designs to maximise the rate of neurite population regrowth.

Keywords

Multidisciplinary; Peripheral Nerve Injury; Descrete-continous model

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Abstract **#793**

NEURAL DIFFERENTIATION OF HUMAN ADIPOSE¬-DERIVED STEM CELLS IN RESPONSE TO ELECTROSPUN FIBROUS GELATIN MATERIALS ENRICHED WITH MAGNETIC NANOPARTICLES AND GRAPHENE OXIDE

Aida Selaru#P[1], Sorina Dinescu#[1], Alexandra Dobranici[1], Elena Olaret[2], Izabela Stancu[2], Marieta Costache[1]

[1] *Department of Biochemistry and Molecular Biology, University of Bucharest, Bucharest, Romania*

[2] *Advanced Polymer Materials Group, University Politehnica of Bucharest, Bucharest, Romania*

Corresponding author's email: aida.selaru@bio.unibuc.ro

Nanocomponents are widely used as bioactive signals in the field of tissue engineering and regenerative medicine. Thus, the incorporation of magnetic nanoparticles (MNPs) and graphene oxide (GO) in the structure of biomaterials proved to have a positive impact on cellular behavior. Here, we analyzed a fibrous composite based on fish gelatin (G), loaded with MNPs (GM), GO (GG) or both (GMG) for its potential to support the neural differentiation of human adipose-derived stem cells (hASCs). After seeding of hASCs, cells were induced towards the neural lineage for 14 days. Detection of expression for specific neural markers (MAP2, Sox2, β-III tubulin and NeuN) was performed using Real-Time PCR and protein expression was detected by immunolabeling coupled with confocal microscopy. Our results showed that the expression levels found on GM and GG were significantly higher than those found on G control material. Interestingly, better results were obtained for GMG scaffold, implying that the presence of both MNPs and GO contributed to the formation of a proper microenvironment for the neural differentiation of hASCs. In conclusion, we demonstrated that the novel nanomaterials are suitable for applications in the field of nervous tissue engineering and further studies are required in order to elucidate the specific molecular mechanisms involved in the neural differentiation of hASCs facilitated by the presence of MNPs and GO.
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Keywords

magnetic nanoparticles ; graphene oxide ; human adipose-derived stem cells

Abstract **#794**

INCREASED VIABILITY AND FUNCTION WITH EXTRACELLULAR MATRIX COMPONENT ENHANCED ALGINATE ENCAPSULATED ENDOTHELIAL, ALPHA AND BETA CELL COMPOSITE AGGREGATES FOR IMPROVED FUNCTION OF MICROCAPSULES UNDER HYPOXIC CONDITIONS

Adam L. Stell$_P$[1], Sami G. Mohommed[2], Fredrik C. Wieland[2], Vijayganapathy Vaithilingam[2], Vanessa L.S. LaPointe[2], Aart A. Van Apeldoorn[2]

[1] *MERLIN Institute, Maastricht University*

[2] *MERLN Institute, Maastricht University*

Corresponding author's email: a.stell@maastrichtuniversity.nl

Transplanting alginate-encapsulated pancreatic islets or beta cells is a promising strategy for the treatment of type 1 diabetes. However, islets used for encapsulation often show lower functionality due partially to islet dysfunction associated with the destruction of the extracellular matrix (ECM) interactions during the islet isolation process. Furthermore, factors produced by alpha and endothelial cells have a great impact on beta-cell insulin secretion. Therefore, mutual signaling between alpha, endothelial cells, and beta cells should be considered for the development of encapsulation systems to achieve high insulin secretion and maintain beta-cell viability. Here, we investigate whether coculture of beta with alpha and endothelial cells could improve beta-cell function within encapsulated aggregates of these cells.Rat insulinoma INS1E, Mouse insulinoma Alpha TC1-6 cells, and human umbilical endothelial cells were used for creating composite aggregates on agarose microwell platform. The composite aggregates were encapsulated within alginate beads with and without extracellular matrix components (collagen IV and laminin 111). Their functionality was assessed by glucose-induced insulin secretion test and compared to encapsulated and non-encapsulated free-floating beta-cell aggregates under hypoxic conditions.Upon glucose stimulation, their insulin secretion is improved in comparison to aggregates consisting of only beta-cells. Moreover, the composite aggregates encapsulated within alginate beads secrete more insulin than aggregates consisting of only beta-cells.Composite aggregates of beta, alpha, and human umbilical vein endothelial cells have improved insulin secretion in comparison to beta-cell aggregates alone showing that the interaction of beta, alpha, and endothelial cells is crucial for a functional encapsulation system.

Keywords

Alginate; islet encapsulation; type I diabetes

Abstract **#796**

RENAL CELL EXTRACELLULAR VESICLES IMPROVE FUNCTIONAL PHENOTYPE OF KIDNEY TUBULOIDS FOR THE USE IN BIOARTIFICIAL KIDNEY

Rafael S Lindoso[P][1,2], Estela M Juncosa[1], Fjodor A Y Yengej[3,4], Maarten B Rookmaaker[4], Marianne C Verhaar[4], Rosalinde Masereeuw[1]

[1] *Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands*

[2] *Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

[3] *Hubrecht Institute-Royal Netherlands Academy of Arts and Sciences, Utrecht, the Netherlands*

[4] *Department of Nephrology and Hypertension, University Medical Centre Utrecht, Utrecht, the Netherlands*

Corresponding author's email: r.soareslindoso@uu.nl

BACKGROUND

The kidney tubuloids (KT), organoids from adult stem cells, are an interesting option for the development of bioartificial kidney (BAK) since they generate functional renal cells. Reproducing complete cell function remains a challenge. Extracellular vesicles (EVs) can regulate several cellular processes, including cell maturation. We aimed to investigate the use of renal EVs as an inducer of drug transporters, as OAT1, in KT and as supporter of tight monolayer formation.

METHODS

EVs from immortalized proximal tubule cells overexpressing OAT1 (ciPTEC-OAT1) were isolated and incubated with KT. As control, conditioned media (CM) of ciPTEC-OAT1, depleted or not of EVs, were used. Gene expression was determined by qPCR and Western blotting. For proximal tubule engineering, hollow fibers were seeded with KT exposed to EVs or CM. Monolayer integrity and cell polarity were analyzed by immunofluorescence.

RESULTS

EVs were capable of increasing the mRNA levels of OAT1 (2.6±0.4-fold, $p<0.04$), with respect to control. EVs contained OAT1 protein and mRNA as cargo, indicating a possible direct transfer to KT. Immunofluorescence showed that KT, seeded on hollow fibers and incubated with EVs, presented a 3D tubular structure with tight monolayer (ZO-1 expression) and cell polarity (apical cilia and Na+/K+-ATPase presence).

CONCLUSION

EVs from ciPTEC-OAT1 improved the expression of drug transporters like OAT1 and support tight monolayers formation on hollow fiber. Further research will explore functional characterization of bioengineered tubules for application in BAK.

ACKNOWLEDGEMENTS

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Abstract **#797**

EVALUATION OF KERATIN RICH ELECTROSPUN NANOFIBROUS SCAFFOLD FOR SKIN TISSUE ENGINEERING

Krishna Dixit$_P$[1], Ragavi Rajasekaran[1], Gayatri Mukherjee[1], Santanu Dhara[1]

[1] *IIT Kharagpur*

Corresponding author's email: krishnadixit021@gmail.com

The present study aims at the fabrication of a bioactive nanofibrous scaffold using keratin (Ker) and polycaprolactone (PCL) blended electrospun matrix with an attempt to have protein-rich composition. The homogenous blend of Ker and PCL was successfully prepared using about 60% Ker that was electrospun. The nanofibrous architecture of electrospun matrices was investigated by FESEM, which evidenced smooth nanofiber morphology with a diameter in the range of 150 nm to 300 nm. Further, the presence of a higher volume percent of Ker facilitated cellular affinity due to its innate property of native peptide cues in the protein molecules. FTIR study confirms the chemical interaction of Ker/PCL and the dispersion of natural protein within the PCL matrix. AFM was performed to evaluate the surface roughness with a value of 0.370µm. Contact angle measurement evidenced ~ 90º corresponding to moderate hydrophilicity owing to the presence of PCL in the blend composition. The swelling study revealed that water absorption of 90% in less than 22 h. In vitro evaluation using human dermal fibroblast (DHF) cells confirms enhanced bioactivity, cytocompatibility, and cell affinity on the matrices with enhanced proliferation and adhesion properties. Further, the Ker/PCL matrix was implanted subcutaneously in the mice model for the assessment of cutaneous wound healing efficacy. It was evidenced by wound closure in 19 days without any further treatment. This indicates the fact that Ker/PCL based nanofibrous matrix imbibe all the potency to serve as a substitute for dermal and support skin tissue engineering.

Abstract **#799**

FUNCTIONALISATION OF PEG-BASED HYDROGELS WITH CHONDROGENIC CUES FOR INJECTABLE 3D MODELS AND 3D DRUG SCREENING PLATFORMS

Nicola C Foster[P][1], Richard JA Moakes[1], Alicia J El Haj[1]

[1] *University of Birmingham*

Corresponding author's email: N.C.Foster@bham.ac.uk

Osteoarthritis (OA) is one of the leading debilitating diseases within the adult population. The avascular nature of cartilage limits its capacity for endogenous repair, therefore, costly and invasive exogenous interventions such as microfracture are often required before total joint replacement. Thus there is an urgent need for new drugs and therapies which can delay disease progression.

Synthetic polymers are an attractive choice for tissue engineering applications, as their chemical and mechanical properties can be controlled and manipulated with relative ease. Poly(ethylene glycol) (PEG) is one such polymer, which has already been widely utilised in the medical field. We have developed the first fully synthetic fluid gel from a PEGDA precursor using a combination of UV curing and the application of shear force2. We have shown that these fluid gels are cytocompatible and, when functionalised with hyaluronic acid (HA), they promote increased expression of SOX9 in a human bone marrow stromal cell (BMSC)-derived cell line. These synthetic microgel suspensions (SyMgels) offer great potential for use as injectable cell therapies for joint repair.

In addition we have developed a 3D model of chondrogenesis using quiescent PEG hydrogels functionalised with chondro-inductive molecules such as HA. Progenitor cells are seeded on top a molecule such as Wnt3a (which induces proliferation, migration and asymmetrical division) and allowed to migrate into the hydrogel where they respond to chondroinductive cues. This model can easily be adapted to a 96/384-well format and used for high throughput screening of potential therapeutic molecules for joint repair.

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Abstract **#800**

CHONDROGENIC DIFFERENTIATION IN THE SPOTLIGHT – USING PHOTOBIOMODULATION TO ENHANCE THE CHONDROGENIC POTENTIAL OF ASC

Conny Schneider$_P$[1,2], Barbara Schädl[3,2], Susanne Wolbank[1,2], Heinz Redl[1,2], Sylvia Nürnberger[1,4,2], Peter Dungel[5,2]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria*

[2] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[3] *University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria*

[4] *Department of Orthopedics and Trauma-Surgery, Division of Trauma-Surgery, Medical University of Vienna, Vienna, Austria*

[5] *Adipose-derived stem/stromal cells (ASC) are gaining attention as therapeutically relevant cells for tissue regeneration and might be a potent tool for cartilage regeneration. Their potential to differentiate into the chondrogenic lineage, however, varies between donors. In our study, we aim to stimulate ASC towards a more reliable chondrogenic phenotype using photobiomodulation (PBM). Human ASC of donors with varying chondrogenic potential were subjected to PBM by LED devices (Repuls) of either blue (475 nm), green (516 nm) or red (635 nm) light, at two different energy densities. The treatment was applied either during the 2D expansion phase or the 3D differentiation phase. After 5 weeks of micromass pellet culture, chondrogenic differentiation was assessed via pellet size, GAG/DNA content, histology and gene expression analysis. We were able to demonstrate that, depending on treatment parameters and intrinsic cellular potential, PBM can be used to activate or deactivate the chondrogenic differentiation of ASC. Reactions to PBM, both positive and negative, were more pronounced when the treatment was applied during expansion. While the donor with the highest intrinsic chondrogenic potential reacted to PBM during expansion with decreased differentiation, chondrogenesis was activated in donors with low potential. Red light was most reliable in inducing chondrogenesis and had the least adverse effects in the donor reacting negatively to PBM. Energy density had little to no influence on the outcome. Thus, by choosing appropriate parameters, PBM offers a fast, cost-effective and non-invasive treatment option to enhance the performance of ASC in cartilage tissue engineering.*

Corresponding author's email: cornelia.schneider@trauma.lbg.ac.at

Adipose-derived stem/stromal cells (ASC) are gaining attention as therapeutically relevant cells for tissue regeneration and might be a potent tool for cartilage regeneration. Their potential to differentiate into the chondrogenic lineage, however, varies between donors. In our study, we aim to stimulate ASC towards a more reliable chondrogenic phenotype using photobiomodulation (PBM).

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Thus, by choosing appropriate parameters, PBM offers a fast, cost-effective and non-invasive treatment option to enhance the performance of ASC in cartilage tissue engineering.

Abstract **#801**

MULTIFUNCTIONAL SURFACES WITH CELL-INSTRUCTIVE AND ANTIBACTERIAL PROPERTIES

Manuel Gomez-Florit[P][1], Adriana Vilaça[1], Hanna Tiainen[2], Rui MA Domingues[1], Barbara B Mendes[1], Alejandro Barrantes[2], Rui L Reis[1], Manuela E Gomes[1]

[1] *3B's Research Group, University of Minho*

[2] *Department of Biomaterials, University of Oslo*

Corresponding author's email: mgflorit@i3bs.uminho.pt

Metallic implants replace hard tissues in orthopedic and dental applications. Many are anchored in the bone and extend through soft tissues, either temporarily or permanently, to aid in recovery from lesion or to replace native tissues.[1] However, a main reason causing their failure is the lack of integration of metallic surfaces with soft tissues. This allows bacteria entering the implantation site, form a biofilm and trigger a complicated inflammatory response.[2] We hypothesized that multifunctional surfaces with cell-instructive and antimicrobial properties could improve soft tissue integration while decreasing bacterial colonization. We created anisotropic nanopatterns based on the controlled deposition of cellulose nanocrystals (CNC) on metallic surfaces. Furthermore, we used CNC surface chemistry to sequester and present bioactive molecules from platelet lysate (PL). The ability of these surfaces to guide cell growth was tested using human gingival fibroblasts. The bioactivity of the nanocoatings was tested using macrophage polarization assay and bacteria live/dead assay. We produced anisotropic nanopatterns on titanium surfaces by spin coating CNC at high speed. Furthermore, CNC-coated surfaces sequestered and formed a coating of PL-derived biomolecules, as confirmed by proteomic analysis. The anisotropic nanopatterns guided fibroblasts growth and alignment for 14 days of culture. Moreover, the PL-derived proteins polarized macrophages towards M2-like phenotype. As well, the PL-coated surfaces showed antibacterial activity against Staphylococcus aureus. These results suggest that the developed multifunctional surfaces could promote soft tissue integration to metallic implants and, at the same time, prevent bacterial invasion, tissue inflammation, and failure of biomedical metallic implants.

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Abstract **#802**

C-MYC PATHWAY MODULATION IN CELL ACTIVATED BY PLATELET LYSATE STIMULATION

Marta Nardini$_P$[1,2], Chiara Gentili[3], Patrizio Castagnola[4], Ranieri Cancedda[5], Maddalena Mastrogiacomo[1,2]

[1] *Biotherapy Laboratory, Department of Internal Medicine (DIMI) University of Genova, Genova, Italy*

[2] *IRCCS Ospedale Policlinico San Martino, Genova, Italy*

[3] *Cellular Oncology Laboratory, Department of Experimental Medicine (DIMES), University of Genova, Genova, Italy*

[4] *Biotherapy Laboratory, IRCCS Ospedale Policlinico San Martino, Genova, Italy*

[5] *Endolife S.r.l., Genova, Italy*

Corresponding author's email: nardinimarta88@gmail.com

Platelet Lysate (PL), containing platelet growth factors, is able to support isolation, expansion and proliferation of Mesenchymal Stem Cells derived from different tissues and cell lines. The PL effect on different types of cells and the molecular mechanisms involved in these processes remains poorly understood. We focused our attention on a family of highly conserved proteins involved in different cellular mechanisms such as cell growth, proliferation and apoptosis: C-MYC's family. The three isoforms of C-MYC are:C-MYC1, C-MYC2 and C-MYCS differently expressed during cell growth. MSCs obtained from Bone Marrow, Adipose tissue, Articular Chondrocyte, human Osteoblast, Amniotic Fluid Stem Cells and HeLa cell line, were cultivated in medium supplemented with 10% FBS or 5% PL for proliferation analysis, apoptosis and C-MYC protein expression.

All primary cell cultures treated with PL showed a high proliferation rate respect to cultures treated with FBS and expressed the C-MYC1 isoform, which was absent when cells were cultured in FBS. The same cells expressed the C-MYC2 isoforms independently from the culture conditions. For the cell line, the C-MYC1 isoforms was expressed also in FBS condition. Immunofluorescence analysis indicated that MSC cultures showing C-MYC1 protein expression were in a proliferative stage. All these experiments showed that PL was responsible for the induction or increasing of C-MYC1 isoform in primary cells culture and much more slightly in the cell line. These results pave the way to a deeper molecular study in order to understand peculiar differences of PL stimulation on various types of cells.

Keywords

Multipotent (mesenchymal) stem cells; C-Myc; Platelet lysate

Abstract **#803**

3D TRIPLE CULTURES OF OSTEOCYTES, OSTEOCLASTS AND OSTEOBLASTS AS IN VITRO BONE MODEL AND ITS RESPONSE TO DEGRADATION PRODUCTS OF RESORBABLE MAGNESIUM ALLOYS

Anne Bernhardt$_P$[1], Heike Helmholz[2], Max von Witzleben[1], Regine Willumeit-Römer[2], Michael Gelinsky[1]

[1] *Centre for Translational Bone Joint and Soft Tissue Research, TU Dresden*

[2] *Helmholtz Centre Geesthacht, Institute of Material Research Metallic Biomaterials*

Corresponding author's email: anne.bernhardt@tu-dresden.de

An in vitro bone model comprising all bone cells (osteocytes, osteoblasts and osteoclasts) arranged in a spatially defined manner was developed. Instead of using cell lines we isolated all cells for the model from primary human tissue. Based on previously developed co-culture systems of human primary osteocytes with osteoblasts (1) and osteoclasts (2) we extended the system to a triple culture of all bone cell species. Osteocytes were differentiated in collagen I gels, spatially separated by porous membranes from osteoblasts and osteoclasts. Silicon templates were used to seed osteoblasts and osteoclasts onto the membrane and to separate the cell species after cultivation for further analysis. All cell types developed their typical morphology, which is dendritic extrusions for osteocytes, multinucleated cells for osteoclasts and cobblestone-like morphology for osteoblasts. Furthermore, specific markers for the different cell species were detected both by gene expression analysis and by measurement of specific enzyme activities like alkaline phosphatase, tartrate resistant acid phosphatase, cathepsin K and carbonic anhydrase II.

Magnesium and its alloys are innovative resorbable biomaterials with potential for application as bone implant, since the mechanical properties are similar to bone. Furthermore, magnesium was shown to stimulate osteogenesis (3). Therefore, triple cultures were cultivated in the presence of degradation products of magnesium materials. We demonstrated changes of gene expression of marker genes for osteocytic and osteoblast differentiation.

In conclusion, the tested in vitro bone model comprised markers of all bone cells and is a useful tool to test bone graft materials, especially those releasing bioactive ions.

Keywords

3D in vitro bone model; osteocyte; resorbable magnesium alloy

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Abstract **#804**

NOZZLE-FREE ACOUSTIC DROPLET EJECTION ENABLES MULTISCALE 3D BIOPRINTING

Stefan Jentsch$_P$[1], Ramin Nasehi[1], Christoph Kuckelkorn[1], Benedikt Gundert[1], Sanja Aveic[1], Horst Fischer[1]

[1] *RWTH Aachen University Hospital*

Corresponding author's email: sjentsch@ukaachen.de

Bioprinting allows the manufacture of complex cell-laden hydrogel constructs that can mature into tissue replacements in a subsequent cell culture process. The nozzles used in currently available bioprinters are critical components because they limit the print resolution. In addition, at dimensions below 100 µm clogging is expected. The reduction of the nozzle diameter also increases shear stress during printing process. At a critical shear stress, mechanical damage to printed cells triggers cell death. To overcome these limitations, a novel 3D bioprinting method based on the principle of acoustic droplet ejection (ADE) is introduced here. The absence of a nozzle in this method minimizes critical shear stress, amplifying the options for bioprinting. A numerical simulation reveals that maximum shear stress during the ADE is 2.7 times lower than with a Ø150 µm microvalve nozzle. Average droplet diameters with a minimum size of 54 µm and a maximum of 183 µm were achieved. Printing of cell clusters contained in droplets at the millimeter length scale, as well as in droplets the size of a single cell is feasible. The precise 3D build-up of cell-laden structures is proven. Evidence is provided that the method has no negative effects on stem cell morphology, proliferation, or differentiation capacities. The multi-scale acoustic bioprinting technique thus holds promise for cell-preserving creation of complex and individualized cell-laden 3D hydrogel structures.

Keywords

innovative bioprinting technology; acoustic droplet ejection; human cells

Abstract **#805**

IN SILICO DESIGN OF ADDITIVELY MANUFACTURED CALCIUM-PHOSPHATE BASED SCAFFOLDS FOR MAXILLOFACIAL APPLICATIONS

Bingbing Liang[P][1], Liesbet Geris[1,2], France Lambert[3], Ehsan Sadeghian[1], Dorien Van Hede[3]

[1] *Biomechanics Research Unit, GIGA In silico Medicin, University of Liege, Belgium*

[2] *Biomechanics Section, KU Leuven, Belgium*

[3] *Dental Biomaterials Research Unit, University of Liège, Belgium*

Corresponding author's email: bingbingliang1102@gmail.com

The use of ceramic biomaterials for dental bone regeneration is a reliable treatment option compared to the autologous bone which involves higher morbidity. Improving scaffold design for optimal osseointegration can be obtained by changes to the local geometry, surface roughness, material composition, etc. In this study, we report the development and use of a computational model describing neotissue formation in 3D scaffolds. After calibration with in vitro experiments, we use the model to design 3D structures that subsequently are tested in a calvarial model in rat.

The level-set method is used to simulate the neotissue growth. [1] For the in vivo study, 2 designs were selected: a lattice structure with square struts and a gyroid structure. BioOss, currently the standard biomaterial used in the clinic, was taken along as a control. The scaffolds were implanted under a CaP shell on the calvaria of rats. Scaffolds were explanted after 2 and 8 weeks, n=10 for per condition per time point, and evaluated using nanofocus Computed Tomography and histology.

The computational model developed in this study was used to design geometries for 3D printed CaP-based scaffolds. In vivo testing of these scaffolds showed a strong improvement of the amount of bone formation in the model-based design (gyroid) over the clinical standard (BioOss) or a classical 3D printed geometry (orthogonal lattice). This result, obtained without several in vivo iterations as normally would need to be performed, clearly shows the added value of in silico tools in the design of novel biomaterial structures.

Keywords

alveolar bone regeneration ; Calcium-phosphate biomaterials; model design

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Abstract **#806**

A NOVEL SILK REINFORCED BIPHASIC 3D PRINTED PEGT/PBT SCAFFOLD FOR OSTEOCHONDRAL TISSUE ENGINEERING

Thomas Braxton$_P$[1], Khoon Lim[2], Cesar Alcala-Orozco[2], Habib Joukhdar[3], Jelena Rnjak-Kovacina[3], Tim Woodfield[2], Lin-Hua Jiang[4], Xiaodong Jia[5], Xuebin Yang[1]

[1] *Biomaterials and Tissue Engineering Group, Dept. of Oral Biology, University of Leeds, WTBB, St. James's University Hospital, Leeds LS9 7TF, UK*

[2] *CReaTE Group, Dept. of Orthopaedic Surgery, University of Otago Christchurch, Christchurch 8140, New Zealand*

[3] *Graduate School of Biomedical Engineering, UNSW Sydney, NSW 2052, Australia*

[4] *School of Biomedical Sciences, University of Leeds, UK*

[5] *School of Chemical and Process Engineering, University of Leeds, UK*

Corresponding author's email: mntbra@leeds.ac.uk

Osteochondral tissue damage is common in the young, active population, remaining a major clinical challenge. 3D printing is an exciting method for development of novel scaffolds for tissue regeneration especially within personalised medicine. However, many 3D printing techniques rely on creating a lattice structure, which often demonstrates poor cell bridging between filaments to fill the gaps due to its large pore size; reducing regenerative capacity as host or seeded cells are unable to efficiently remodel the scaffold. This study aimed to tackle this issue by developing a novel silk reinforced 3D printed scaffold. Biphasic scaffolds consisted of 3D printed poly(ethylene glycol)-terephthalate-poly(butylene-terephthalate)(PEGT/PBT) lattice, infilled with a cast and freeze dried porous silk scaffold, along with a seamless silk top layer. Fluorescent microscopy showed biphasic scaffolds and silk scaffold controls can support human bone marrow stromal cell attachment and spreading after 24hours of seeding with no obvious difference. No clustering of cells was seen around the 3D printed lattice in the biphasic scaffold. Compression testing showed that scaffolds had a compressive modulus of 12.7±0.9Mpa and ultimate compressive strength of 1.56±0.1Mpa, theoretically allowing for the scaffold's survival during implantation and joint articulation without stress-shielding mechanosensitive cells. The silk material provides a secondary structure to the 3D printed scaffold enhancing cell attachment and bridging. The 3D printed scaffold provides a solid framework for the biphasic scaffold and increases its strength and versatility. These results indicate that the novel silk reinforced biphasic 3D printed scaffold is biocompatible and has suitable properties for osteochondral tissue regeneration.

Keywords

3D printing ; Osteochondral; scaffold

Abstract **#808**

DESIGN AND DEVELOPMENT OF ARTIFICIAL NERVE CONDUIT

Thanusha AV_P[1], Maurice N Collins[1]

[1] *STOKES LAB, BERNAL INSTITUTE, UNIVERSITY OF LIMERICK, IRELAND*

Corresponding author's email: Thanusha.av@ul.ie

Development of artificial nerve or nerve substitute from biopolymers will ease the requirements for donor nerve autograft and plays an effective role in the treatment of peripheral nerve injuries. The major disadvantage of commercially available nerve substitutes is lack of conductivity, failed to repair large nerve gap defects, limited availability and they are expensive to procure. The present research work is focussed on the fabrication of an artificial nerve conduit using the gelatin-based porous structure. Bioactive components such as glycosaminoglycans, curcumin were suffused in to the nerve conduit to enhance the native nerve action. Physicochemical characterization studies include morphology, swelling, degradation, tensile strength, conductivity test, Attenuated total reflection Fourier transform infrared analysis (ATR-FTIR), Differential Scanning Calorimetry (DSC) and Thermogravimetric analysis (TGA). Bio-based porous nano-carbon fibres were incorporated as a conducting material to the optimized nerve conduit to develop an electroconductive artificial nerve. In-vitro cytocompatibility studies include cell viability, cell proliferation assays and Immuno-fluorescence staining of protein markers on neural stem cells. The acquired result demonstrates that the artificial nerve has characteristic features like ease of scalability, cost-effective ,biodegradable, biocompatible and effective for the treatment of peripheral nerve injuries.

Keywords

Artificial nerve; Biodegradable; Electro-conductive

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Abstract **#809**

SONICATION AIDED HIERARCHICAL TAILORING OF ZNO/AG NANOPARTICLES ON A NATURAL SUBSTRATE TOWARDS ENHANCED WOUND REGENERATION

Preetam Guha Ray[P][1], Shreya Biswas[2], Piyali Basak[2], Santanu Dhara[1]

[1] *Biomaterials and Tissue Engineering Laboratory, School of Medical Science and Technology (SMST), Indian Institute of Technology Kharagpur, India*

[2] *School of Bioscience and Engineering, Jadavpur University, Kolkata-700032, India*

Corresponding author's email: preetamguharay@gmail.com

Silver nanoparticles (Ag-NP) have long been deployed for wound regeneration process owing to its high antibacterial efficacy however fabrication of an effective delivery matrix with enhanced efficacy is still an unresolved challenge. The study demonstrates a unique route to tailor a nanobiocomposite (EAZ) with effective loading of Ag–NPs wherein eggshell membrane (ESM) acted as a natural substrate and zinc oxide (ZnO) nanoparticles were deployed to attain a three dimensional spatial hierarchy for decoration of the Ag-NPs. A simple sonochemical guided approach was adopted to in-situ decorate ZnO nanoflakes and Ag-NPs on top of the microfibrous ESM in order to an attain sustain release profile. Microstructural analysis ratified hierarchically spatial arrangement of Ag–NPs on the three dimensional ZnO nanoflakes anchored on microfibrous eggshell membrane. XPS analysis confirms presence of Ag-NPs in its metallic state and also justify interaction between ZnO and the Ag–NPs. Further, ICP-MS studies substantiate excellent and sustainable release profile of Ag-NP from the nanobiocomposites at varying pH. The nanobiocomposites demonstrated exceptional bactericidal activity against Gram-negative, E. coli or P. aeruginosa and Gram-positive, S. aureus or B. subtilis bacterial cells. The nanobiocomposite exhibited excellent cytocompatibility when seeded with Human dermal fibroblast (HDF) cells. Furthermore, when grafted over a full thickness wound model, EAZ samples presented fast re-epithelization and collagen deposition thus leading to complete wound closure in 12 days. The architectural tribology along with exceptional wound regeneration kinetics of the nanobiocomposites manifests its application as an alternate platform in wound regeneration applications.

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Abstract **#810**

BONE-TARGETING DELIVERY OF ALENDRONATE FOR THE TREATMENT OF OSTEOPOROSIS.

Shen-Mao Chen[P][1,2], Lingling Zhao[3], Tung-Chun Lee[4], Chaozong Liu[1]

[1] *Division of Surgery and Interventional Science, University College London, UK*

[2] *Department of Orthopaedic Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*

[3] *Ningbo University*

[4] *Institute for Materials Discovery, University College London, UK*

Corresponding author's email: shen-mao.chen.16@ucl.ac.uk

BACKGROUND & AIMS:

Osteoporosis is a major health burden. Current therapeutic treatments have disadvantages such as systemic side effect and low bioavailability. Bone-targeting drug delivery system are designed to improve the therapeutic effect of drugs and minimize the potential toxic side effects. We fabricated a novel drug nanocarrier for bone-targeting alendronate delivery using glycol chitosan (GC)-poly(lactide-co-glycolide) (PLGA) and PLGA-alendronate conjugates.

MATERIALS & METHODS:

Chitosan-based nanoparticles were prepared with GC-PLGA and PLGA-alendronate conjugate by nanoprecipitation. Alendronate sodium, a commonly used bisphosphonate drug for osteoporosis therapy, serves as both a bone-targeting ligand and a loading drug. The size of the nanoparticles was determined by dynamic light scattering (DLS) measurement. The morphology of the GC-PLGA/PLGA-alendronate nanoparticles was examined by scanning electron microscopy (SEM). Drug release profile, cytotoxicity and cellular uptake were evaluated in vitro. Bone-targeting potential was assessed by hydroxyapatite binding assay and an ex vivo porcine bone model.

RESULTS:

The conjugation of GC-PLGA and PLGA-alendronate was confirmed by Fourier-transform Infrared Spectroscopy (FTIR) and nuclear magnetic resonance (NMR) analysis. The prepared nanoparticles are highly aqueous dispersible with an average size range from 90 to 130 nm based on various polymer ratio. Morphological characterization (SEM) revealed that the nanoparticles are spherical in shape. In vitro tests demonstrated sustained drug release of alendronate, good biocompatibility and intracellular uptake of nanoparticles. Hydroxyapatite affinity test and ex vivo porcine bone model confirmed the bone-targeting potential.

CONCLUSION:

The bone-targeting GC-PLGA nanoparticles may be a drug delivery system for the treatment of osteoporosis. Further study of biodistribution is warranted.

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Abstract **#812**

FUCOIDAN HYDROGELS SIGNIFICANTLY ALLEVIATE OXIDATIVE STRESS AND ENHANCE THE ENDOCRINE FUNCTION OF ENCAPSULATED BETA CELLS

Vijayaganapathy Vaithilingam[P][1], Lara L Reys[2], Mireille MJPE Sthijns[3], Timo Rademakers[3], Rick de Vries[1], Sami J Mohammed[1], Denise de Bont[1], Marlon Jetten[1], Eelco de Koning[4], Nizar I Mourad[5], Pierre Gianello[5], Simone S Silva[2], Rui L Reis[2], Tiago H Silva[2], Aart van Apeldoorn[1]

[1] Cell Biology Inspired Tissue Engineering (CBITE), MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands

[2] 3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4805-017 Barco, Guimarães, Portugal

[3] Department of Complex Tissue Regeneration (CTR), MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands

[4] Faculty of Medicine, Leiden University Medical Centre, Leiden University, The Netherlands

[5] Pôle de Chirurgie Expérimentale et Transplantation, Université Catholique de Louvain, Brussels, Belgium

Corresponding author's email: v.vaithilingam@maastrichtuniversity.nl

Background: Oxidative stress is a major factor limiting encapsulated islet efficacy. Fucoidans (marine polysaccharide) possess strong anti-oxidant properties (1) but its effects on islet encapsulation is unknown. We assessed the feasibility of fucoidan hydrogels (Fucogel) for islet encapsulation to improve viability and function.

Methods: Fucogel prepared by blending fucoidan with alginate and fucoidan microcapsules made using a droplet generator. Total antioxidant capacity (TAC) and viscoelastic properties of Fucogel assessed. Ability of Fucogel to alleviate oxidative stress determined using transduced INS1E cells. Human, rat and neonatal pig islets encapsulated in Fucogel and viability, ATP and insulin secretion assessed. Fucogel potential to protect islets against cytokine and H2O2 induced oxidative damage assessed.

Results: The TAC of Fucogel was ~35-fold higher compared to alginate hydrogel. Both alginate and Fucogel had similar viscoelastic properties with no differences in their hydrogel mesh sizes. Fucogel significantly alleviated intracellular oxidative stress in transduced INS1E compared to alginate. Viability and ATP levels of human islets in Fucogel at days 1 and 5 were significantly higher compared to those in alginate. Human islets in Fucogel functioned better in response to high glucose with a ~ 2-fold higher stimulation index compared to alginate. Similar results were seen with rat and neonatal pig islets encapsulated in Fucogel. Exposure to proinflammatory cytokines or H2O2 dramatically rescued human islets in Fucogel with significantly higher viability, ATP and stimulation index compared to those encapsulated in alginate.

Conclusion: Fucoidan hydrogels possess strong antioxidant property, significantly alleviate oxidative stress and enhance encapsulated islet viability and function.

Keywords

Fucoidan hydrogel; Microencapsulation; Oxidative stress

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Abstract **#813**

INFLUENCE OF STATINS ON CARTILAGE HOMEOSTASIS AFTER TRAUMATIC LOADING AND DURING CHONDROGENIC DIFFERENTIATION

Jana Riegger-Koch$_P$[1], Sai Pulasani[1], Rolf Brenner[1]

[1] *Division for Biochemistry of Joint and Connective Tissue Diseases, Department of Orthopedics, University of Ulm, Ulm, Germany*

Corresponding author's email: jana.riegger@uni-ulm.de

As inhibitors of HMG-CoA-reductase, statins are widely used as cholesterol-lowering drugs. Due to the anti-inflammatory effects, statin therapy has also been discussed for osteoarthritic disease. Therefore, we investigated the potential therapeutic effects of fluvastatin (hydrophile) and simvastatin (lipophile) after ex vivo cartilage trauma. Macroscopically intact human cartilage was obtained with consent of donors undergoing total knee joint replacement (n=11). Cartilage explants were subjected to a blunt trauma (0.59 J) using a well-established drop tower system and treated with simvastatin or fluvastatin (1 µM, 5 µM and 10 µM) for 7 d. Afterwards, cell viability (live/dead assay) as well as gene and protein expression of collagen type II (COL2) and catabolic enzymes (MMP-2, -13) were determined. Moreover, the influence of statins during chondrogenic re-differentiation of dedifferentiated chondrocytes was investigated (Saf-O staining; COL2 IHC). Statistics: one-way ANOVA.

Cell viability was decreased by 20.2 % after cartilage trauma. Both statins exhibited concentration-dependent cell protection and anti-catabolic effects as demonstrated by reduced expression of MMP-13 as well as attenuation of latent and active MMP-2. Chondroprotection was also reflected in decreased breakdown of COL2. While both statins suppressed the gene expression of COL2, only simvastatin had inhibitory effects on the biosynthesis. However, re-differentiation of dedifferentiated chondrocytes was impaired by both statins. Although, the tested statins exhibited cell and chondroprotective potential after ex vivo cartilage trauma, the therapeutics also suppressed chondroanabolic processes. Nevertheless, statin therapy might have beneficial effects during the acute phase after joint injury, reducing cell death and enzymatic cartilage destruction.

Keywords

statins; cartilage trauma; chondroprotection

Abstract **#814**

A NOVEL MULTIPHASE 3D IN VITRO MODEL TO STUDY THE BONE-TENDON-MUSCLE INTERFACE DEVELOPMENT

Wendy Balestrip[1], Rob Morris[2], John Hunt[3,4], Yvonne Reinwald[1]

[1] Department of Engineering, School of Science and Technology, Nottingham Trent University, Nottingham, UK

[2] Department of Physics and Mathematics, School of Science and Technology, Nottingham Trent University, Nottingham, UK

[3] Medical Technologies and Advanced Materials, School of Science and Technology, Nottingham Trent University, Nottingham, UK

[4] College of Biomedical Engineering, China Medical University, Taichung 40402, Taiwan

Corresponding author's email: wendy.balestri2017@my.ntu.ac.uk

Regeneration and function of musculoskeletal tissues have been extensively investigated. Within that however, less focus has been placed on the interfaces between them. These transition zones may not regenerate after severe damage or deterioration, leading to incomplete patient recovery. The interfaces and composition are well known physiologically, but the knowledge on their development is limited1, especially for the tendon-muscle interface.

This research has developed a 3D in vitro model to investigate bone-tendon-muscle interface development. A mould was designed, and 3D printed in resin, containing different surface topographies; analogous to pores for bone and ridges mimicking fibres for tendon and muscle. Collagen-agarose composite gels were solidified in the mould. A gradient in stiffness was established by changing concentrations of collagen type I and agarose; and varying concentrations of hydroxyapatite. Human osteoblast-like cells (MG-63), human dermal fibroblast (HDF) and human skeletal muscle cells (Sket.4U) were co-cultured in the 3D model. Different cell seeding densities were tested (5,000, 50,000 and 100,000 cells/gel), to determine the optimum seeding densities for cells to reach homeostasis. To evaluate this point, cell metabolic activity, viability and proliferation were measured. The optimum cell seeding densities were 50,000 cells/gel for MG-63, as proliferation and metabolic activity were stable between day 7 and 14 ; and 100,000 cells/gel for Sket.4U and HDF, because, even though the DNA content decreased between day 7 and 14, the metabolic activity was stable (HDF) or increased over time (Sket.4U). Moreover, a unique 3D construct, consisting of different materials, mechanical properties and topographies was developed.

Keywords

Interfaces; Regeneration; Indirect 3D printing

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Abstract **#815**

DEVELOPMENT OF SULFOBETAINE POLYMER CONJUGATES AS A DRUG DELIVERY SYSTEM TO CANCER CELLS AGGREGATES

Masaya Yamamoto$_P$[1], Nobuyuki Morimoto[1]

[1] *Tohoku University*

Corresponding author's email: masaya.yamamoto.b6@tohoku.ac.jp

Cell aggregates have attracted much attention in the research field of drug screening and regenerative medicine as a mimicry of in vivo microenvironments in culture. However, the poor permeability of substances, such as drugs and nutrients, is a big challenge in using cell aggregates. We developed a random copolymer composed of a sulfobetaine monomer, 3-dimethyl(methacryloyloxyethyl)ammonium propane sulfonate (DMAPS) and a macromonomer of poly(ethylene glycol) methacrylate (PEGMA), P(DMAPS-PEGMA), as a fast intracellular drug delivery. In this study, we investigated the permeation, growth suppression, and invasion inhibition of anticancer drugs-conjugated P(DMAPS-PEGMA) in tumor cell aggregates. Doxorubicin (Dox) and 17-AAG were selected as anticancer drugs and conjugated with P(DMAPS-PEGMA), (Mn = 17,000). Tumor cell aggregates of human glioblastoma A-172 cells or human hepatoma HepG2 cells were prepared from 1,500 cells/well in a low protein adhesion 96-well plate by 4-day cell culture. The fluorescence of Dox-P(DMAPS-PEGMA) was detected in the center of the tumor cell aggregates after 1-2 h incubation under confocal laser scanning microscope, while Dox alone could permeate through only 2 to 3 cell layers from the outside. Dox-P(DMAPS-PEGMA) significantly suppressed the growth of tumor cell aggregates. However, the growth suppression was a little bit lower than that of Dox alone. On the contrary, 17-AAG-P(DMAPS-PEGMA) exhibited more than ten times higher inhibition than that of 17-AAG alone in the A-172 cell invasion from the tumor cell aggregates in Matrigel. These results suggest that conjugation with sulfobetaine copolymers allows anticancer drugs to permeate throughout tumor cell aggregates, leading to enhance the drug efficacy.

Abstract **#816**

A BIOPRINTED MODEL FOR METASTASIS OF TUMOUR CELLS INTO A HEALTHY BONE-LIKE REGION

Megan Cooke$_P$[1,2], Michael Weber[2], Derek Rosenzweig[2]

[1] *Department of Surgery, McGill University*

[2] *Division of Orthopaedics, McGill University*

Corresponding author's email: megan.cooke.research@gmail.com

A staggering 96% of oncology drugs fail to reach FDA approval and enter the market, one reason being the challenges of evaluating such drugs in physiologically relevant culture systems. This is exacerbated within bone metastasis, where spread of tumour cells to bone is often fatal. The complex bone tumour microenvironment is host to a range of biochemical and physical cues which cannot be replicated in 2D in vitro culture. In this study, bioprinting was used to produce a 3D model of tumour cell migration into a bone-like region. Two bioinks were prepared: 1) healthy primary human osteoblasts in an alginate-gelatin-hydroxyapatite blend was bioprinted into a ring shape before 2) MDA-MB-231 breast cancer cells were printed into the central cavity using an alginate-gelatin ink. Models were then cultured in the presence of doxorubicin, a common chemotherapeutic. The migration of tumour cells into the outer 'healthy' region of the model was quantified using light-sheet imaging, before quantifying the effect of tumour cell invasion and doxorubicin treatment on the ability of osteoblasts to form bone-like matrix. Results showed that without doxorubicin treatment mineralised bone-like matrix was formed in the outer 'healthy' region. Tumour cells were then shown to migrate through the alginate-gelatin matrix into the outer region over 28 days. Increasing the doxorubicin concentration reduced formation of multi-cellular tumour spheroids and thus, decreased migration of tumour cells into the healthy bone-like region. The bioprinted interface model therefore may be suitable for screening novel therapeutics against tumour growth and migration.

Abstract **#817**

OPTICAL BIOSENSORS FOR GLYCATED ALBUMIN DETECTION

Himadri Shekhar Mondal[P][1], Krishnan Murugappan[2], Adam Damry[3], Colin Jackson[3], Antonio Tricoli[2], David Nisbet[1]

[1] Laboratory of Advanced Biomaterials, Research School of Engineering, ANU College of Engineering & Computer Sciences, The Australian National University, Canberra 2601, Australia.

[2] Nanotechnology Research Laboratory, Research School of Engineering, ANU College of Engineering & Computer Sciences, The Australian National University, Canberra 2601, Australia.

[3] Research School of Chemistry, Australian National University, Canberra 2601, Australia.

Corresponding author's email: himadrishekhar.mondal@anu.edu.au

Biosensors are devices engineered to detect important biomarkers by generating analytical signals based on biological or chemical reactions. Here, we have engineered an optical biosensor based on localized surface plasmon resonance (LSPR) that can detect low concentrations of Glycated Albumin (GA). GA is an important biomarker for Diabetes that provides an indication of glucose level history over a roughly three week period in diabetes patients helping to track the overall effectiveness of Diabetes management strategies.. Gold nanoparticles were deposited on a quartz substrate by flame synthesis, a scalable, and low-cost synthetic route. These substrates were conjugated with a DNA aptamer capable of binding GA resulting in a plasmonic wavelength shift due to binding of GA to the DNA aptamer. This prototype biosensor was able to detect micromolar concentrations of GA. In the future, improvements to sensor architecture are expected to increase the sensitivity and selectivity.

Abstract **#818**

THE INFLUENCE OF SUBCHONDRAL BONE ON CARTILAGE IN OSTEOARTHRITIS

Majoska Berkelaar$_P$[1,2], Alessandro Pirosa[2], Sebastian Müller[3], Ivan Martin[2], Andrea Barbero[2]

[1] *Department of Biomedical Engineering, University of Basel, Switzerland*

[2] *Department of Biomedicine, University Hospital Basel, University of Basel, Switzerland*

[3] *Orthopedics department, University Hospital Basel, Switzerland*

Corresponding author's email: majoska.berkelaar@unibas.ch

Introduction Osteoarthritis (OA) is a debilitating disease of the entire joint characterized by degeneration of cartilage and altered bone remodeling with no available cure. The cause of OA remains unknown and the role of the subchondral bone is largely unstudied. Therefore, this study aims to identify OA subchondral bone factors that can induce phenotype changes in chondrocytes.

Methods Tibial plateaus were harvested from OA patients undergoing knee arthroplasty. Mass spectroscopy-based proteomics screening was performed on the conditioned medium (CM) of sclerotic and non-sclerotic OA bone, to identify factors secreted by the latter. Furthermore, chondrocytes isolated from intact and damaged OA cartilage regions were cultured for two weeks in a polyHEMA-coated 96 well plate. The resulting micro-cartilages were characterized biochemically, histologically, and by RT-PCR at the end of chondrogenic culture and after stimulation with sclerotic and non-sclerotic OA bone CM.

Results Proteomics revealed 108 significantly differentially expressed proteins between sclerotic and non-sclerotic OA bone CM, including POSTN, WIF1, and ALPL. Micro-cartilages generated with damaged OA chondrocytes expressed increased catabolic markers, including MMP13, ADAMTS4-5, and lower GAG amounts compared to those generated with intact OA chondrocytes. After stimulating the micro-cartilages with the OA CM, the expression of MMP13 increased compared to chondrogenic culture.

Conclusion The secretome of sclerotic and non-sclerotic OA bone is significantly distinct as determined by proteomics. The micro-cartilage model captures catabolic traits of OA chondrocytes from different degenerative states and will be used for experiments to study the influence of specific bone factors on chondrocyte alterations in OA.

Keywords

Bone; Cartilage; Osteoarthritis

Abstract **#819**

BIOMIMETIC SCAFFOLD - ABM/P-15 FOR IMPROVING BONE REGENERATION SPEED AND EFFICACY: FROM DISCOVERY TO COMMERCIALISATION

Xuebin Yang$_P$[1], Yamuna Mohanram[1], Jingying Zhang[1,2], Eleftherios Tsiridis[3], Scott Johnson[4]

[1] *University of Leeds*

[2] *The Second Clinical Medical College, Guangdong Medical University*

[3] *Aristotle University Medical School*

[4] *Cerapedics, Inc.*

Corresponding author's email: denxy@leeds.ac.uk

To date, i-FACTOR™ Peptide Enhanced Bone Graft is the only osteobiologic to utilise the biological activity of a 15-amino acid peptide sequence (P-15) for stimulating the natural, self-regulating bone healing process, resulting in safe, predictable bone formation. P-15 is responsible for cell attraction, attachment, activation and initiation of the cascade of events leading to bone matrix formation. The combination of an osteoconductive anorganic bone mineral (ABM) scaffold and P-15 peptide provides a 3D biomimetic environment to support cellular attachment and early bone formation. The mechanism of action is attachment mediated, thereby reducing the risk of unwanted ectopic bone formation. Our results have found that P-15 promotes human bone marrow stromal cells (HBMSCs) and human dental pulp stromal cells (HDPSCs) adhesion and growth on the ABM scaffold and also increases the expression of alkaline phosphatase, the formation of Type I collagen matrix and calcium accumulation in vitro and leads to early bone matrix formation in vivo. The combination of HDPSCs with ABM-P-15 can improve bone regeneration speed and efficacy. Increased regulatory scrutiny needs for robust translational science and high-quality clinical evidence. The core ABM/P-15 technology has been used in dentistry in over 500,000 patients (PepGEN P-15, Dentsply Inc.) and in orthopaedic and spinal applications (i-FACTOR™) which have over 100,000 cases. In 2015 i-FACTOR™ became the first biologic bone graft to receive U.S. PMA approval for use in anterior cervical decompression/fusion (ACDF). The Level I randomised study concluded that i-FACTOR™ was significantly superior over the control, autograft bone.

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Abstract **#820**

DERIVATION OF FUNCTIONAL HEPATOCYTES FOR LIVER TISSUE ENGINEERING.

Inchirah Adala$_P$[1], Jopeth Ramis[1], Andrew Bennett[1], Cameron Alexander[1], Felicity Rose[1]

[1] *University of Nottingham*

Corresponding author's email: Inchirah.ADALA1@nottingham.ac.uk

Liver disease represents one of the fastest-growing diseases in the world, with an increasing Liver diseases represent one of the fastest-growing disease classes in the world, with an increasing mortality in the UK of 400% since the 1970s.1 Developing in vitro liver models to understand the underlying disease mechanisms is crucial for future treatments. Limited access to primary liver tissue, reduced survival time and the inability of primary hepatocytes to proliferate in culture had led researchers to look for an alternative cell source from which to secure a supply of mature, functional hepatocytes. 2 In severe liver injury, bipotential hepatocyte progenitor cells (HPC) located next to the bile duct are able to proliferate and subsequently differentiate into either hepatocytes or cholangiocytes. 3 In this study, we isolate human EPCAM positive HPC, expand them as organoids, and drive their differentiation and maturation on a 3D substrate. A 3D coaxial electrospun scaffold has been developed in order to mimic some of the biochemical features of the liver ECM environment and to deliver a combination of signalling molecules to aid in cell differentiation and maturation. The use of a synthetic electrospun scaffold in our model allows us to provide a more defined environment for cell expansion in comparison to 2D culture systems such as matrigel.

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Abstract **#821**

3D BIOPRINTING OF STEM CELL SPHEROIDS DISPERSED IN A POLYSACCHARIDE-BASED HYDROGEL FOLLOWED BY EFFICIENT POST-PRINTING CHONDROGENIC DIFFERENTIATION

Monize Caiado Decarli[P][1,2], Adrián Seijas Gamardo[2], Paul Wieringa[2], Jorge Vicente Lopes da Silva[3], Ângela Maria Moraes[1], Lorenzo Moroni[2], Carlos Mota[2]

[1] School of Chemical Engineering, University of Campinas - UNICAMP, Cidade Universitária "Zeferino Vaz", Av. Albert Einstein, 500; 13083-852 – Campinas-SP/Brazil

[2] MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, Universiteitssingel, 40 – Maastricht, The Netherlands

[3] Three-Dimensional Technologies Nucleus, CTI Renato Archer, Rodovia Dom Pedro I SP-65, Km 143,6; 13069-901 - Amarais – Campinas-SP/Brazil.

Corresponding author's email: m.caiadodecarli@maastrichtuniversity.nl

Spheroid-based bioprinting uses uniform spherical cell aggregates as building blocks to manufacture tissue constructs. Tissue maturation is more advanced in spheroids compared to isolated cells and a much higher concentration of cells can be precisely deposited. This offers the possibility of obtaining more complex and larger tissues, through controlled and reproducible ways. However, efficient spheroid-based bioprinting is still challenging, especially regarding construct stability during long periods of the maturation process. Assessing this approach to obtain mature cartilage tissue is of high interest, since it presents a low self-repair capability. Here, we optimized a set of parameters to improve the bioprinting process aiming at establishing a high throughput method to obtain stable spheroid-based constructs. First, we produced a high amount of uniform human mesenchymal stromal cells (hMSCs) spheroids using a micro-molded non-adhesive hydrogel. Spheroids encapsulated in a xanthan gum/alginate hydrogel were bioprinted and the structure ionically crosslinked. A high number of multilayered bioprinted hMSC spheroid-based constructs could be obtained. Thirty days post-bioprinting the constructs were still stable and cell viability in the spheroids high, with profuse extracellular matrix production. The cells in the construct were efficiently differentiated using chondrogenic media for 28 days, and expression of glycosaminoglycans and collagen II was clearly observed. Results on the quantification of specific chondrogenic markers will be also presented. Overall, we hypothesize that these results show the potential of an effective approach to obtain spheroids-based constructs to be applied in the biofabrication of cartilage tissue, as well as models of cartilage diseases studies.

Keywords

spheroid-based bioprinting; bioprinting; cartilage tissue

Abstract **#822**

SOUND WAVES AND BIOMATERIALS TO RECREATE THE EXTRACELLULAR MATRIX

Anne Géraldine Guex[P][1], Nicola Di Marzio[1], Mauro Alini[1], Tiziano Serra[1]

[1] *AO Research Institute Davos, Switzerland*

Corresponding author's email: geraldine.guex@aofoundation.org

Recent efforts in the field of Tissue Engineering have increasingly considered bottom-up approaches and the assembly of living and non-living building blocks into functional tissues. A particularly interesting aspect in this regard is remote control over the spatial organisation of cells or cell-spheroids within a carrier matrix by use of acoustic fields. This method takes advantage of acoustic waves and their generated hydrodynamic forces that act on cells to arrange them within a matrix. Importantly, however, viscosity and gelation kinetics of the precursor matrix, as well as mechanical integrity of the final hydrogel have only been addressed to a limited extent.

Here, we are exploiting the potential of acoustic manipulation at low frequencies (40 to 200 Hz) to pattern beads and subsequently cells in biomaterials of various origin, concentration, and viscosity. We experimentally investigate the relationship of the resistive force $F=3\pi\eta dv$ (where η is the viscosity of the matrix and v is the velocity of the cell) and compare the resulting patterns to a model of particle dynamics. This force, imposed by the hydrogel precursor, needs to be overcome by the hydrodynamic force induced by the applied frequency and amplitude in order to move cells within the matrix. Better insight into the biomaterials' rheological properties will allow fine-tuning of cell patterning and controlled spatial organisation. Our findings will contribute to a better understanding of the underlying effects of acoustic waves in various hydrogels and allow future researchers to tune the viscosity of their materials in order to achieve successful patterning.

Abstract **#823**

PRE-CLINICAL RANDOMIZED AND CONTROLLED ASSESSMENT OF AUTOLOGOUS AND ALLOGENEIC BIOPRINTED SKIN IN FULL-THICKNESS PORCINE WOUNDS

Adam M Jorgensen[P][1], Anastasiya Gorkun[1], Kelsey Willson[1], Cara Clouse[1], Naresh Mahajan[1], Justin Campbell[1], Garrett Briggs[1], Sean Murphy[1], Sang Jin Lee[1], James Yoo[1], Shay Soker[1], Anthony Atala[1]

[1] *Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC, 27101.*

Corresponding author's email: ajorgens@wakehealth.edu

Current tissue engineered skin fails to meet the need for skin replacement in full-thickness wounds. Bioprinting technology allows for organization of multiple cell types into biomimetic layers. In prior studies, we demonstrated that bioprinted skin integrates, vascularizes, and forms an epidermal barrier when implanted into mice. The purpose of this study is to assess the feasibility and efficacy of autologous and allogeneic bioprinted skin to treat full-thickness wounds in pigs. Porcine keratinocytes, fibroblasts, and pre-adipocytes were isolated from skin punch biopsies and endothelial cells from a carotid artery biopsy. Cells were expanded in vitro, suspended in bioink, and bioprinted to form a biomimetic tri-layer skin construct. 5x5cm excisional full-thickness wounds were made and treated with autologous bioprinted skin, allogeneic bioprinted skin, autograft, allograft, or left without treatment. Treatments sites were randomized, with blinded surgery and analysis. Digital photographs are taken twice per week during bandage changes for digital planimetry analysis, and serum proteins from bandages are used for proteomic analysis. The pigs are euthanized on day 28, and tissue samples are analyzed by histology, SEM, and omics analysis. We expect improved wound closure in autologous bioprinted skin treated wounds, improved epidermal maturity, and increased blood vessel density compared with the other treatments. Also, we expect normal collagen organization in wounds treated with autologous bioprinted skin. These results will suggest integration of the autologous bioprinted skin into the wound to support skin regeneration and healing. Ultimately, this technology could translate into a new treatment for full-thickness wounds in human patients.

Keywords

Skin regeneration; Wound healing; Skin engineering

Abstract **#824**

MIMICKING THE INTERVERTEBRAL DISC MICROENVIRONMENT FOR EXPANSION OF NUCLEUS PULPOSUS PROGENITOR CELLS IN A CONTEXT OF CELL THERAPY

Julien Guerrero[P][1], Sonja Häckel[2], Andreas S. Croft[1], Christoph E. Albers[2], Benjamin Gantenbein[1,2]

[1] Tissue Engineering for Orthopaedics & Mechanobiology (TOM), Department for BioMedical Research (DBMR) of the Medical Faculty of the University of Bern, University of Bern, Bern, Switzerland.

[2] Department of Orthopaedic Surgery& Traumatology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland.

Corresponding author's email: julien.guerrero@dbmr.unibe.ch

Increasing evidence implicates intervertebral disc (IVD) degeneration as a significant contributor to low back pain. In this respect, tissue-specific progenitors may play a crucial role in tissue regeneration. Recently, a progenitor cell population (Tie2+) was described in the nucleus pulposus (NP) tissue of the IVD (1). Here, we studied the influence of the culture method and of the microenvironment on the human NP progenitor's proliferation and their differentiation potential in vitro.

The NP cells (NPCs) were obtained from trauma patients. Briefly, the NPCs were cultured in 2D (monolayer) or 3D (alginate beads) conditions. After one week, cells from 2D or 3D culture were expanded on fibronectin-coated flasks. Subsequently, expanded NPCs were then characterised by tri-lineage differentiation. Moreover, experiments using pure Tie2+ and Tie2- NPC populations were also performed, following the same study protocol.

In this study, we observed that only 3D culture of NPCs was able to maintain pluripotent gene expression. Moreover, only NPCs in 3D culture presented stromal cell surface markers. We were also able to maintain the expression of Tie2 marker in 3D culture overtime in our NPC populations.

The present study aims to demonstrate that 3D expansion of NP cells increases the differentiation potential compared to 2D culture. Our results highly suggest that the maintenance of pluripotent capacity is mainly but not exclusively due to the higher presence of Tie2+ cells. This project, by evaluating the influence of a two-step expansion protocol on the functionality of NP progenitors could also lead to an innovative clinical approach.

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Abstract **#826**

A CONTROLLED AND REPRODUCIBLE APPROACH TO OBTAIN CELL SPHEROIDS OF ADULT AND EMBRYONIC HUMAN STEM CELLS

Monize Caiado Decarli[P][1], Mateus Vidigal[2], Mariana Harue Taniguchi Nagahara[1], Rafael Abboud Azoubel[1], Julia Adami Nogueira[3], Alexandre Leite Rodrigues de Oliveira[2], Jorge Vicente Lopes da Silva[3], Ângela Maria Moraes[1]

[1] School of Chemical Engineering, University of Campinas - UNICAMP, Cidade Universitária "Zeferino Vaz", Av. Albert Einstein, 500; 13083-852 – Campinas-SP/Brazil

[2] Laboratory of Nerve Regeneration, Institute of Biology, University of Campinas - UNICAMP, Cidade Universitária "Zeferino Vaz", Rua Monteiro Lobato, 255; 13083-862 - Campinas-SP/Brazil

[3] Three-Dimensional Technologies Nucleus, CTI Renato Archer, Rodovia Dom Pedro I SP-65, Km 143,6; 13069-901 - Amarais – Campinas-SP/Brazil.

Corresponding author's email: m.caiadodecarli@maastrichtuniversity.nl

Three-dimensional spherical cellular aggregates can effectively mimic the natural microenvironment of tissues and organs, consisting of a feasible alternative for many different purposes. Recently, approaches involving sophisticated engineered strategies aiming to control spheroid diameter and shape, to reduce cell shear stress and to promote efficient mass transfer, are of high interest. The objective of this work was to evaluate the performance of an optimized device we have been developing over the years to obtain spheroids through controlled and reproducible ways. The methodology is based on the use of a micro-molded non-adhesive hydrogel. First, we designed a positive primary multiple well mold (Rhinoceros CAD) with previously defined well geometry, which was manufactured by 3D Printing (Polyjet Technology, Stratasys). The mold was used to cast a negative hydrogel structure in which human dental pulp stem cells (hDPSC) and fluorescent human embryonic stem cells (hESC) were cultured in alfaMEM and mTeSR™1 respectively, for 7 days to form the spheroids. Successful spheroid formation in large quantities was attained, around one-thousand spheroids in a single six well-plate. The spheroids showed appropriate shape and size distribution. Spheroids of hDPSC and hESC, starting from an inoculum 7,100 cells per spheroid, presented mean diameters around 250 and 500 micrometers, respectively. Fluorescence microscopy analysis showed presence of live cells in the whole 3D structure of hDPSC and hESC spheroids. Overall, these results show that 3D culturing stem cells with our improved method allows obtaining easily a large number of uniform and viable spheroids.

Keywords

Spheroid; Cell aggregation ; 3D cell model

Abstract **#828**

DEVELOPMENT OF A POLYMERIC HEART VALVE FOR MINIMALLY INVASIVE AORTIC VALVE REPLACEMENT

Aida Cavallo$_P$[1,2], Emanuele Gasparotti[3], Paola Losi[2], Ilenia Foffa[2], Tamer Al Kayal[2], Emanuele Vignali[3], Simona Celi[3], Giorgio Soldani[2]

[1] *Sant'Anna School of Advanced Studies, Institute of Life Sciences,Italy, Pisa*

[2] *Institute of Clinical Physiology, National Research Council, Massa, Italy*

[3] *BioCardioLab, Fondazione Toscana "G. Monasterio"Heart Hospital, Massa, Italy*

Corresponding author's email: aida.cavallo@ifc.cnr.it

Minimally invasive techniques for valve replacement (MIAVR) are the common therapeutic approach that motivates the search for new valve materials[1]. In this study, a polymeric self-expandable tri-leaflet heart valve compatible with MIAVR procedure is designed, fabricated and characterized. The valve is composed by a polymeric stent to support the polymeric leaflets and an external metallic stent for anchoring on the aortic root.

The polymeric stent was fabricated by 3D printing technology using the CarboSil 55D, a thermoplastic silicone-polycarbonate urethane with good biocompatibility and hemocompatibility[2,3]. The valve leaflets were fabricated using dipping and custom spray-machine apparatus to spray a CarboSil 80A 2,5% solution in tetrahydrofuran/dioxane 1:1[4].

The external frame was made in nickel-titanium by laser cutting to obtain a self-expandable valve after the crimping process. The elements were assembled in the completed device using a surgical suture and characterized: i) assessing mechanical properties of the leaflets, ii) crimping test, iii) fatigue test and iv) evaluating hemodynamic parameters of the valve.

The employed technologies enable the fabrication of sprayed CarboSil leaflets with reproducible mechanical properties. The proposed design provides a self-expandable and crimpable device able to reduce its initial diameter up to 40% and to restore its initial dimension without shape changes. The fatigue test showed no leaflets macroscopic damages after 5x10^6 cycles and the hemodynamic performances were confirmed: the Effective Orifice Area and the total regurgitation fraction indexes showed values within the reported standard ranges for aortic valve (ISO5840-2).

Therefore, further in-vivo studies are required for assessing the long-term valve performance.

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Abstract **#829**

MODELING BONE MARROW IN LIQUIFIED CAPSULES: A NEW THERAPEUTIC STRATEGY FOR BONE REGENERATION

Cláudia S Oliveira$_P$[1], Clara R Correia[2], João F Mano[2]

[1] *CICECO, University of Aveiro*

[2] *CICECO, University Of Aveiro*

Corresponding author's email: claudiao@ua.pt

Stem cell-based bone tissue engineering has emerged as a promising approach for regenerating critical-size bone defects (1). Considering the repair of bone fracture a complex biological mechanism that requires the recruitment of stem cells from the bone marrow (BM), this microenvironment and its components become a powerful candidate to facilitate bone regeneration. This work used mesenchymal stem cells (MSC), hematopoietic stem cells (HSC), and human umbilical vein endothelial cells (HUVEC) to developed multifunctional BM in liquified capsules. Before the encapsulation, aggregates of MSC and surface-modified polycaprolactone microparticles (µPCL) were performed under osteogenic stimulation. Multilayered and liquified alginate capsules were produced as described (2,3). Tri-culture capsules (aggregates, HSC, and HUVEC) and co-culture (aggregates and HUVEC) were maintained in static and dynamic culture systems (spinning flasks). We observed an increase in cell viability and the expression of bone formation markers such as osteopontin, osteocalcin, and bone morphogenetic protein 2 (BMP-2) in both types of capsules cultured in the dynamic system compared to the static, but more significant in tri-culture capsules. The presence of vascular endothelial growth factor (VEGF) was also more prominent in the tri-culture capsules. Our results evidence the superior biological outcome resulting from the direct crosstalk between the BM cell components in the tri-culture capsules. The model supports cell viability, the secretion of osteo key-components, and vasculogenic potential. Our multifunctional liquified capsules mimicking the BM can provide an advanced platform of fundamental importance for new bone regeneration applications.

Keywords

bone marrow; liquified capsules; bone regeneration

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Abstract **#832**

DEVELOPMENT OF AN OPEN SOURCE 3D BIOPRINTING DATABASE FOR EXTRUSION PRINTING

Bhushan Mahadik[P][1,2], Anthony Melchiorri[3,2], Sang Jin Lee[4,2], James Yoo[4,2], Anthony Atala[5,2], Antonios Mikos[6,2], John Fisher[1,2]

[1] *University of Maryland*

[2] *NIBIB/NIH Center for Engineering Complex Tissues*

[3] *Rice University*

[4] *Wake Forest Institute for Regenerative Medicine*

[5] *Wake Forest Institute for Regenerative Medicine*

[6] *Rice University*

Corresponding author's email: mahadikb@gmail.com

3D printing and bioprinting has witnessed a phenomenal rise in the fields of tissue engineering and regenerative medicine over the past few years. Due to the nascent nature of this field, there exists a broad knowledge base regarding various 3D printable material formulations and their unique printing conditions. This poses a challenge for researchers who wish to apply new or established 3D printing techniques and want a reliable source of information regarding 3D printing conditions. At the NIH/NIBIB Center for Engineering Complex Tissues (CECT), we have developed the world's first public repository/database of articles involving 3D printing in the field of tissue engineering and regenerative medicine that are easily searchable by a user. The database is free for use and can be found at https://cect.umd.edu/3d-printing-database. Key printing parameters are displayed, including bioink and cell composition, pressure, temperature, speed, and crosslinking methods. The database also allows us to draw insights into material printability and lays a foundation for machine learning approaches to further optimize multi-material 3D printing. We have created an open survey (https://umdsurvey.umd.edu/jfe/form/SV_ahIl2SK4amMiIIJ) that allows users to enter their published information regarding their 3D printing and bioprinting conditions, which is then added to this database. The ultimate aim is to make this database an active and reliable resource for the 3D printing and bioprinting community. While the current database is limited only to extrusion-based 3D printing, we hope to add other printing techniques such as stereolithography, inkjet printing and direct write electrospinning in order to reach a broader biofabrication audience.

Keywords

Database; 3D printing

Abstract **#834**

FIBRIN-SPECIFIC SHORT LINEAR PEPTIDE MOTIF

Yoon Sung Nam[P1], Moon Young Nam[1], Jeong Heon Yu[1]

[1] KAIST

Corresponding author's email: yoonsung@kaist.ac.kr

The excess formation of fibrin networks, called hypercoagulation, is an important cardiovascular disease biomarker. However, it is technically very challenging to specifically diagnose hypercoagulation because fibrin is structurally very similar (~80 %) to its precursor, fibrinogen, which is abundant in the blood. Here we report a fibrin-targeting heptameric peptide specifically binding to the pocket space of fibrin generated during the fibrinogen-to-fibrin transformation. Fibrinogen is a 340 kDa plasma glycoprotein that facilitates blood clotting and has three submodules (alpha, beta, and gamma) in its soluble form. According to the crystal structure of the gamma module, pocket space is generated when P2 (γ381-390) in the beta-sheet structure is exposed outward and interact with adjacent P2. Computational docking simulations are used to design a heptameric peptide (IPLVVPM) that can bind to the pocket space where the P2 moiety is pulled out. The computational analysis indicates that the peptide including the PxxxP linear motif and amino acids with a hydrophobic and small side chain (e.g., isoleucine, valine, and leucine) can be inserted into the generated pocket space. For the experimental confirmation of the interaction between the peptide and the pocket space of the fibrin gamma module, two variations of recombinant proteins are generated: full gamma module and P2-truncated gamma module. The specific binding of the selected peptide to the pocket space is analyzed using recombinant variants of the gamma module by size exclusion chromatography and circular dichroism.

Abstract **#835**

BIOARTIFICIAL" POLYMER NANOPARTICLES FOR EFFICIENT OLIGONUCLEOTIDE RELEASE FOR DISEASE TREATMENT AND/OR TISSUE REGENERATION

Marco Campisi[1], Marcello Aramu[1], Sharon Lee[2,3], Giulia Adriani[2,3], Enrico Patrucco[4], Claudia Voena[4], Roberto Chiarle[4,5], Clara Mattu[6], Valeria Chiono[6]

[1] *Dept. of Mechanical and Aerospace engineering, Politecnico di Torino, Turin, Italy*

[2] *Singapore-MIT Alliance for Research and Technology (SMART), Singapore, Singapore*

[3] *Singapore Immunology Network (SIgN) Agency for Science, Technology and Research (A*STAR) Singapore, Singapore*

[4] *Dept. of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy*

[5] *Dept. of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, US*

[6] *Dept. of Mechanical and Aerospace engineering, Politecnico di Torino, Turin, Italy*

Corresponding author's email: marco.campisi@polito.it

Oligonucleotides (e.g. siRNAs, microRNAs) and synthetic cyclic dinucleotides have enormous potential for the treatment of diseases (e.g. c ancer) and for tissue regeneration. However, they suffer from poor stability in physiological fluids and limited cell uptake [1]

In this work, "bioartificial" polymeric nanoparticles (NPs) for oligonucleotide encapsulation were prepared by nanoprecipitation from chitosan (CS) and poly(lactic-co-glycolic acid) (PLGA).

Nanocomplexes between positively charged CS and negatively charged oligonucleotides (microRNA mimic: miR-1) were initially formed. Then, PLGA was nanoprecipitated in the presence of CS/miR-1 nanocomplexes, to form "bioartificial" NPs, able to shield payload from degradation, enhance intracellular delivery and reduce cytotoxicity. "Bioartificial" PLGA/CS NPs were prepared with 140 nm hydrodynamic size and surface zeta potential increasing from -20 mV to +30 mV with increasing CS amount, efficiently encapsulating ~99% miR-1. NPs were stabilized against aggregation by adding Tween 80, a polyethylene glycol (PEG)-based emulsifier that helps in reducing protein corona formation, increasing NP stability overtime. Viability assay, performed using human umbilical vein endothelial cells after 24 and 48 h, demonstrated cytocompatibility of NPs at 0.25 - 0.5 mg/mL concentration.

Flow cytometry analysis of 293T cells treated with NPs encapsulating Fluorescein amidites (FAM)-labelled miRNA or CY3-labelled siRNA control revealed high level of transfection efficiency (80%), similar to Lipofectamine® (90%). Confocal imaging confirmed NPs internalization by cells. Overall, the work evidenced that PLGA/CS NPs represent promising candidates for the encapsulation and release of miRNAs and siRNAs, deserving further investigation using therapeutic payloads.

Activity was supported by MIT-POLITO grant (BIOMODE - Compagnia di San Paolo).

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Abstract **#836**

INJECTABLE, SELF-HEALING MESOPOROUS SILICA NANOCOMPOSITE HYDROGELS WITH IMPROVED MECHANICAL PROPERTIES

Aygul Zengin[P][1], João Pedro Olim Castro[1], Pamela Habibovic[1], Sabine van Rijt[1]
[1] *Instructive Biomaterials Engineering, Maastricht University-MERLN Institute, Maastricht, Netherlands*
Corresponding author's email: a.zengin@maastrichtuniversity.nl

Inspired by the autonomous healing process of natural tissues, self-healing hydrogels have received great attention to replacing the often brittle hydrogels currently investigated in clinical applications. However, despite the efforts in the design and development of injectable, self-healing hydrogels, the ongoing challenge is combining high material strength with rapid self-healing. Incorporation of nanoparticles within hydrogels to create nanocomposites is an attractive approach to create hydrogels with improved mechanical and/or biological performance. In particular, mesoporous silica nanoparticles (MSNs) are ideal building blocks within these constructs as they are bioactive, improve mechanical and biological properties of polymers, and can controllably release various types of cargo(1-4).
Here, we report injectable, self-healing, and stimuli-responsive nanocomposites for tissue regeneration using MSNs and hydrophilic linkers that self-assemble in a 3D matrix. In these constructs, the MSNs acted as dynamic covalent crosslinkers in the polymeric network, which resulted in a significant increase of mechanical properties compared to adding nanoparticles as fillers. In addition, their mechanical properties could be tuned by changing MSN weight %, where an up to a 25-fold increase in mechanical properties could be observed compared to the pure polymeric hydrogels. Moreover, these hydrogels degraded completely in a glutathione-containing environment for over six weeks. Additionally, their drug release behavior could be controlled by changing network stiffness. Actin/nuclei staining of encapsulated hMSCs inside the nanocomposites confirmed their biocompatibility. The proposed nanocomposite formulation is a promising strategy to overcome current constraints with the brittleness of self-healing hydrogels, widening their application in tissue engineering applications towards stiffer tissues.

Keywords
Nanocomposite hydrogels; Self-healing; Mesoporous Silica Nanoparticles

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Abstract **#837**

SPACE TANGO'S AUTONOMOUS CELL CULTURE CAPABILITIES FOR BIOFABRICATION AND STEM CELL RESEARCH APPLICATIONS IN MICROGRAVITY

Bill McLambP[1], Paul Kuehl[1], Zach Jacobs[2], Jason Rexroat[2], Gentry Barnett[1], Twyman Clements[1], Jana Stoudemire[1], Andrew Diddle[1], Nikki Leonard[1]

[1] *Space Tango*

[2] *Faradine Systems*

Corresponding author's email: drosales@spacetango.com

Space Tango is recognized as a leader in automated R&D and manufacturing systems for biomedical applications in low-Earth orbit (LEO). The unique domain of space provides a new frontier for discovery and innovation, and our vision is to inspire, innovate and create a better future for humanity, using the microgravity environment of the International Space Station (ISS). Space Tango's emphasis on automation is exemplified by the TangoLab, a fully autonomous system on the ISS since 2016, which allows multiple experiments to run simultaneously and independently on orbit. The recent addition of the Powered Ascent Utility Locker (PAUL) has enhanced Space Tango's on-orbit capacity for cell culture. The PAUL extends the capabilities for environmental control, media exchange, and data collection during the period of ascent and transport to the ISS. The foundational elements of Space Tango's autonomous research and manufacturing systems are compact, smart containers called CubeLabs™. The Cell Culture CubeLab was developed for the study of stem cells, 3-dimensional tissue models, and organoids in microgravity. Incubation and refrigeration subsystems maintain optimal conditions for biological specimens, while the fluid control subsystem provides precision delivery and exchange of media and reagents. The compact imaging system can support brightfield, phase contrast, and 3-channel fluorescence microscopy for high-resolution, live-cell imaging in real-time. Collectively, these integrated subsystems are controlled by the CubeLab flight computer to yield a compact microgravity research tool, toward the development of both capabilities and infrastructure for a new biomedical market sector in the expanding space economy.

Abstract **#838**

MODERATE DEFORMATION OF CARTILAGE MICROPELLETS IN A FLUIDIC CUSTOM-MADE DEVICE ENHANCES THE EXPRESSION OF CHONDROCYTE MARKERS

Noémie Petitjean[1,2], Marie Maumus[1,3], Gilles Dusfour[2], Patrick Cañadas[2], Christian Jorgensen[1,3], Pascale Royer[2], Simon Le Floc'h[2], Danièle Noël[1,3]

[1] *IRMB, Univ Montpellier, INSERM, CHU Montpellier, Montpellier, France*

[2] *LMGC, Univ Montpellier, CNRS, Montpellier, France*

[3] *Clinical Immunology and Osteoarticular Disease Therapeutic Unit, Department of Rheumatology, CHU Montpellier, France*

Corresponding author's email: noemie.petitjean@umontpellier.fr

Cartilage engineering approaches are being developed for evaluating the repair of articular cartilage lesions but few of them have reached the clinics. One limitation of current approaches is the lack of mechanical stimulus, which is one main factor regulating tissue homeostasis. We therefore aimed at better understanding the impact of mechanical stimulation on cartilage generation and extracellular matrix production. We relied on the use of a fluidic custom-made device for mechanical stimulation and characterization of mesenchymal stromal cells (MSCs)-derived cartilage micropellets. Human MSCs were differentiated into chondrocytes by culture in micropellets with a chondrogenic medium for 21 days. Six micropellets were placed into the conical wells of the chamber of the device and stimulated with different square signals of positive pressure (amplitude, frequency, duration). The sinking of each micropellet into the cone was recorded by a camera and its deformation was analyzed using a finite element model. 24 hours after stimulation, micropellets were harvested for chondrocyte marker quantification (SOX9, AGG and COL2B) by RT-qPCR and for histology. A single stimulation of 30 min with an amplitude of 3.5 kPa superimposed to a minimum pressure of 1.75 kPa, at 1 Hz for 30 min increased the expression of chondrocyte markers. The finite element analysis indicated moderate deformation of micropellets with compression, tension and shear that did not alter micropellet microstructure as shown by histological staining. Our data demonstrate the interest of fluidic-based compression for reproducible stimulation of cartilage micropellets and set the basis for further longitudinal studies on the long term.

Keywords

Mesenchymal stem cells; Micropellets; Mechanical stimulation

Abstract **#839**

THE EGGSHELL MEMBRANE: APPLICATIONS IN REGENERATIVE MEDICINE AND DRUG DEVELOPMENT

Rosemond A Mensah$_P$[1], Kawther Salim[1], Seung-Bin Jo[2], Hoon Kim[2], Sung-Min Park[2], Kapil D Patel[2], Kyong Jin Cho[2], Laura E Sidney[3], Decio Alves-Lima[4], Hungyen Lin[4], Mandakhbayar Nandin-Erdene[2], Jung-Hwan Lee[2], Hae-Won Kim[2], David Y Chau[1]

[1] *Eastman Dental Institute, University College London, London, WC1X 8LD, UK*

[2] *Institute of Tissue Regeneration Engineering, Dankook University, Cheonan 31116, Republic of Korea*

[3] *Academic Ophthalmology, Division of Clinical Neuroscience, School of Medicine, University of Nottingham, Nottingham, UK*

[4] *Department of Engineering, Lancaster University, Lancaster, LA1 4YW, UK*

Corresponding author's email: d.chau@ucl.ac.uk

The chicken eggshell membrane (ESM) is an exceptional biomaterial demonstrating unique biological characteristics and defined mechanical/physical properties. As such, the ESM has potential to be exploited as a novel membrane for regenerative therapy or as a biomimetic scaffold for advanced cell culture. In this study, a number of techniques were employed to extract (and enhance) the ESM. Thereafter, the membrane was characterized in terms of its physical, mechanical and biological properties.

ESM samples were prepared using either a manual, an optimised acid- and/or chelator-based extraction protocol and the physical/mechanical characteristics were assessed using DMA, SEM, WCA, FT-IR and THz sensing. Additional crosslinking of the ESM was introduced using EDC-NHS and an enzymatic technique. In vitro assessment was performed using standard biological assays for cell attachment/spreading, viability and mitochondrial activity with human (melanoma) fibroblast cell lines (Malme-3/Malme-3M), immortalised human corneal epithelial cells (ihCEC) and cornea mesenchymal (stromal) stem cells (cMSC). The chicken embryo chorioallantoic membrane (CAM) assay was also exploited to determine the effect of the ESM on angiogenesis.

Complete ESM samples were successfully isolated, modified and thereafter fully characterized. Cells cultured on the extracted ESM demonstrated high biocompatibility in terms of high cell attachment, spreading, viability and proliferation rates/characteristics and also allowed the promotion of new blood vessels (i.e. pro-angiogenic) in the CAM assay. As such, this work summarizes the development of an ESM-based material/scaffold- modulation of the characteristics of the membrane can be achieved via the isolation/extraction/preparation/enhancement techniques used and, therefore, be adapted to suit its final translational application.

Keywords

eggshell membrane; natural; dressing

Abstract **#840**

NANO-ANALYTICAL CHARACTERIZATION OF METAL ORGANIC FRAMEWORK BIOTRANSFORMATION

Anna Neuer$_P$[1,2], Alexander Gogos[1], Alexandre Anthis[1,2], Inge Herrmann[1,2]

[1] Empa - Laboratory for Particles-Biology Interactions, Department of Materials Meet Life, Swiss Federal Laboratories for Materials Science and Technology (Empa), Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland.

[2] ETH Zurich - Nanoparticle Systems Engineering Laboratory, Institute of Process Engineering, Department of Mechanical and Process Engineering, ETH Zurich, Sonneggstrasse 3, 8092 Zurich, Switzerland.

Corresponding author's email: annalena.neuer@empa.ch

Metal-organic-frameworks (MOF) are a rapidly growing class of nano-porous networks, with an extremely high (internal)-surface area. They find application in storage, separation and purification fields. Because of their tailorability, MOFs hold promises in the biomedical field as drug delivery agents, radioenhancers and application in theranostic and personalized medicine. For biomedical applications, the fate of these new materials is, next to efficacy, a major determinant of clinical success. Compared to their metal oxide counterparts, MOF typically exhibit higher instability and different degradation/biotransformation behaviours.

Zeolite-imidazole-framework-8 nanoparticle (ZIF-8) are one of the most extensively studied nanomedical MOFs.(1) Next to application as drug carrier with anti-inflammatory and anti-microbial properties(2) a ZIF-8-chitosan hydrogel composite for bone regeneration with angionesis and osteogenesis promoting properties was recently presented.(3) However, clinical translation is still challenging, amongst other reasons a tight control over nanomaterial chemistry during nanomedical life-cycle is needed. Stability and dissolution was excessively studied in the past but a detailed understanding of behaviour and chemical reaction in-vivo leading to phasetransformation and/or generation of secondary nanomaterial hampering efficacy, elimination and potential side effects is widely unexplored.

To overcome the gap between material engineering and medical application, and enable more rationalized safe-by-design material engineering, we investigate the environment-dependent biochemical-transformation of MOFs in physiologically relevant liquids and in-vitro systems. We present an example of MOF biotransformation where secondary particle formation with changes in elemental distribution and morphology suggesting nanomaterial formation from degradation products. Taken together, our nano-analytical characterization approach offers a route to follow the biological fate of MOF-based na-nomedicines.

Keywords

Metal-Organic Frameworks; Biotransformation

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Abstract **#844**

ELECTROSPUN SYNTHETIC POLYMERS APPLIED TO THE GENERATION OF EPIDERMAL EQUIVALENTS

Luiz Henrique Catalani$_P$[1], Denisse Esther Mallaupoma Camarena[1], Larissa Sekimoto[2], Silvya Stuchi Maria-Engler[2]

[1] *Institute of Chemistry, University of São Paulo*

[2] *School of Pharmaceutical Sciences, University of São Paulo*

Corresponding author's email: catalani@usp.br

The reduction of animal testing and the need to assess the health risk of new chemicals makes tissue engineering an essential tool in the development of in vitro models. In this work, we developed an inexpensive reconstructed human epidermis (RhE) by using electrospun scaffolds from synthetic polymers, like PET, PBT, and Nylon 6/6, and described in terms of its morphology. Epithelial tissues were constructed with and without dermis, followed by its histological characterization by H&E staining and immunohistochemistry, as well as Western Blot analysis. Finally, the epithelial constructions that demonstrated viable structures of RhE were submitted to an applicability model as a platform for skin irritation and corrosion testing, according to standards of OECD TG 431 and 439 [1]. The morphological and immunohistochemistry analyses revealed the presence of a stratified tissue, showing the presence of cytokeratin 14, cytokeratin 10, involucrin, and loricrin arranged in a stratification process, similar to human skin. To proceed with OECD protocols, acetic acid and lactic acid were used as corrosive substances, while SDS and KOH were tested as irritants. The relative viability values obtained for each chemical treatment fully compared with commercial models, as well as with the USP-RHE model [2], demonstrating its usability. These results showed that electrospun synthetic scaffolds are promising three-dimensional structures in the construction of epidermal equivalents.

Keywords

epidermal equivalent; electrospinning; synthetic polymers

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Abstract **#845**

ALIGNED POLYDROXYALKANOATE BLEND FIBRES FOR PERIPHERAL NERVE REPAIR

Caroline Sarah Taylor$_P$[1]

[1] *The University Of Sheffield*

Corresponding author's email: c.s.taylor@sheffield.ac.uk

INTRODUCTION: Nerve guide conduits (NGCs) are a favourable alternative to autograft use for nerve repair. However, as a hollow tube, they lack natural guidance cues for the regenerating axon. The addition of guidance scaffolds, in NGCs, has been shown to increase nerve regeneration distances1. Polyhydroxyalkanoates (PHAs) can be tailored to a specific application and exhibit excellent biological properties.

METHODS: P(3HB) and P(3HO) were produced by bacterial fermentation and characterised2. Aligned PHA and PCL fibres were fabricated by electrospinning, and fibre alignment and diameter quantified. NG108-15 neuronal cells, and primary Schwann cells, were seeded onto scaffolds, for 6 days, and labelled for β III tubulin and S100β. Whole rat Dorsal Root Ganglion (DRG) explants were placed onto fibres in a 3D ex vivo fibre model3. After 21 days, DRGs were labelled for β III tubulin, and S100β, and imaged to determine average neurite length and average Schwann cell migration distance.

RESULTS: Significantly longer neurite lengths were detected on 8µm P(3HB):P(3HO) 50:50 fibres (137.94 ± 17.35µm) compared to the other fibre conditions. However, significantly longer neurites grew from DRGs when cultured on the 5µm P(3HB):P(3HO) 50:50 fibres (1502.69 ±110.63µm). Average Schwann cell migration distance was also significantly higher on 5µm P(3HB):P(3HO) 50:50 fibres compared to 5µm PCL fibres.

DISCUSSION & CONCLUSIONS: Blends of PHAs can be fabricated into aligned fibre scaffolds using electrospinning, with known fibre diameters, for peripheral nerve applications. The use of PHAs eliminates concerns with the use of PCL, and has excellent properties to improve nerve regeneration.

Keywords

nerve ; repair; regeneration

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Abstract **#846**

SCREENING OF CELL CULTURE SUBSTRATES FOR THE EXPANSION OF CORNEAL CELLS IN VITRO

Nello Formisano$_P$[1], Gozde Sahin[1], Rhiannon Grant[1], Roman Truckenmuller[1], Stefan Giselbrecht[1]

[1] *MERLN - Maastricht University*

Corresponding author's email: n.formisano@maastrichtuniversity.nl

Current corneal treatments mostly rely on transplantation of corneas from deceased donors. However, this procedure can only fulfil less than 2% of the global demand. On the one hand, tissue engineering strategies based on a pre-expansion of corneal cells in vitro would allow the treatment of millions of people whose corneas are damaged by trauma or disease. On the other hand, primary keratocytes and endothelial cells of the cornea are notoriously challenging to be maintained in culture or expanded without significantly alter their phenotype. Numerous attempts aim to overcome these issues. We hypothesized that also for corneal cells, physical and architectural cues provided by micro engineered cell culture substrates can be used together with media formulations to better tune cell growth, proliferation and differentiation.
In this study, we investigated the influence of topographically patterned films on the maintenance of native corneal cells phenotype in vitro. We compared several polymeric materials for the fabrication of thin textured films. In addition, we systematically screened synthetic 2 ½ D microtopographies by varying patterns shape, size and height to explore how these affect cell differentiation. We finally analyzed marker expression and cell morphology features to find significant correlations between substrates and cell phenotype. Our results show how a more comprehensive bioengineering approach can contribute to the development of better differentiation protocols for the expansion and differentiation of primary cell cultures in vitro.

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Abstract **#847**

HUVECS ORGANIZATION ON TUNABLE DYNAMIC COVALENT ALGINATE HYDROGELS

Rebeca Rivero[1], Francis Morgan[1], Shahzad Hafeez[1], Agustina Aldana[1], Lorenzo Moroni[1], Matthew Baker[1], Julia Fernández-Pérez$_P$[2,1]

[1] *Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University*

[2] *MERLN Institute, Maastricht University*

Corresponding author's email: j.fernandezperez@maastrichtuniversity.nl

Vascularization is a critical barrier to clinical application and scale of engineered tissues. The fate of engineered constructs after implantation highly relies on the rapid and sufficient vascularization, which guarantees long-term function and survival at the defect area. While some naturally-derived materials, e.g. Matrigel, show promising HUVECs organization these materials are limited by lot-to-lot variability and the inability to precisely tune material properties. In the search for suitable hydrogels to encourage vascularization, controllable and biomimetic systems are need to study the effect of materials properties on HUVECs organization.Here, we utilized a dynamic covalent hydrogel, which has controllable stiffness and stress relaxation, to investigate the response and organization of HUVECs to these mechanical properties, using oxidized alginate, modified with RGD, with different cross-linkers to obtain materials with different storage moduli (G', 13530.7 to 248.8 Pa) and time of relaxation(τ,134205 to 795 s). Rheological characterization revealed that the values of G' and τ of the soft dynamic hydrogels (248.8 Pa and 795 s) are in the same order than the values showed by Matrigel, 11.2 Pa and 755 s. All formulations showed good cytocompatibility. On the soft dynamic hydrogels, we observed cord-like HUVECs organization. Stiffer hydrogels did not show this organization. The HUVECs are being studied in order to determine cell-cell interactions and cell migration.This work contributes to the increasing interest in developing extracellular matrix-like hydrogels to study complex cell-cell and cell-matrix interactions. These soft and hydrogels remain one of the few synthetically controllable systems to show promising HUVECs organization.

Keywords

Dynamic Hydrogels; HUVECs

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Abstract **#848**

STRETCH-GROWN GALSAFE® AXONAL TRACTS SERVE AS LIVING TISSUE ENGINEERED NERVE GRAFTS FOR PERIPHERAL NERVE REPAIR

Kritika Katiyar[P][1], Justin Burrell[2], Franco Laimo[2], Zarina Ali[2], Robert Shultz[2], Kevin Browne[2], John Bianchi[3], Harry Ledebur[1], D. Kacy Cullen[2,1]

[1] *Axonova Medical*

[2] *University of Pennsylvania*

[3] *Revivicor, Inc.*

Corresponding author's email: kritikak@pennmedicine.upenn.edu

Peripheral nerve injury (PNI) often results in protracted denervation of muscle, leading to loss of function. The current gold standard for nerve repair, the autologous nerve graft, not only requires sacrificing healthy nerve necessitating a second surgery with increased risk for donor site morbidity but frequently leads to inadequate functional recovery for large gap injuries. To address this, Axonova has developed tissue engineered nerve grafts (TENGs) by implementing the strategy of axon stretch growth that occurs during development. TENGs are living three-dimensional nerve constructs consisting of longitudinally aligned axon tracts spanning discrete neuronal populations. Based on preclinical data, TENGs target multiple neuroregenerative mechanisms, providing viable alternatives for current repair strategies. For clinical translation, a xenogeneic product strategy is being pursued through a partnership with Revivicor, Inc. Revivicor has developed a genetically engineered strain of pig that does not express a key antigen – galactose-α1,3-galactose (gal) – that would otherwise activate the complement system following transplant into humans, resulting in rapid rejection. Axonova's product development strategy is to build TENGs using neurons from Revivicor's GalSafe® pigs, presenting several advantages: (1) an off-the-shelf gal-free product; (2) cGMP manufacture from a qualified, FDA compliant starting biomass; (3) source of terminally differentiated motor and sensory neurons for modality-specific products. Pre-clinical studies have shown that GalSafe® TENGs promote host axon regeneration through the previously discovered mechanism of axon facilitated axon regeneration (AFAR). Successful biomanufacturing of TENGs is a critical first step in introducing this technology to the clinic as a revolutionary approach to major PNI.

Keywords

Nerve Regeneration; Xenogeneic Cells; Axon Tracts

Abstract **#849**

THE CLOSED SYSTEM PROVIDING READY-TO-USE AVAILABILITY OF CRYOPRESERVED MULTIPOTENT MESENCHYMAL STROMAL CELLS ON CONTACT LENSES FOR OPHTHALMIC USE

Yuriy Petrenko[P][1], Eliska Vavrinova[1], Irena Vackova[1], Sarka Kubinova[1]
[1] *Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic*
Corresponding author's email: yuriy.petrenko@iem.cas.cz

The multipotent mesenchymal stromal cells (MSCs) may promote corneal regeneration through the paracrine action, reducing the inflammatory processes and providing trophic support for the host tissue. To ensure the fast availability of the safe cellular therapy for these applications, several aspects were considered in the study: a) the choice of the polymeric contact lenses able to act as cell carriers; b) the choice of minimal DMSO concentration ensuring sufficient cell viability during cryopreservation; c) the design of closed system, ensuring the seeding of cryopreserved cells onto the contact lenses in safe clinically-relevant environment.
Human Wharton's jelly MSCs were obtained according to ethical guidelines and expanded in the presence of 5% of platelet lysate (PL). The cryopreservation (-1°C/min) was prepared in PL, supplemented with 1-10% DMSO. The cell recovery was assessed by alamar blue assay. The commercially available contact lenses were tested as a carrier for MSCs. The closed system was designed and printed using 3D Creality Ender 3 printer, using PLA filaments.
We found that among the wide range of polymeric contact lenses, only a few types ensured attachment and growth of MSCs. The recovery of MSCs after cryopreservation in PL-based medium varied depending on the DMSO concentration and reached more than 80% at 5% DMSO. The designed closed system allowed to seed cryopreserved MSCs onto the contact lenses, minimizing additional manipulations and reducing the risks associated with contamination. Therefore, the presented approach can be applied for the preparation of therapeutic MSC-based grafts for clinical ophthalmology.
Grant support: EATRIS LM2018133

Keywords
Closed system ; Biopreservation; Contact lenses

Abstract **#850**

METABOLIC MAPPING OF CELL DEATH IN PRIMARY CULTURES AND HYPOXIA-INDUCED DAMAGE IN A PANCREAS-ON-CHIP SYSTEM

Daniel Alejandro Carvajal Berrio[P][1,2], Aline Zbinden[1], Max Urbanczyk[1], Shannon L. Layland[1], Mariella Bosch[1], Sandro Fliri[1], Chuan-En Lu[1], Abiramy Jeyagaran[1], Peter Loskill[1,3], Garry P. Duffy[4], Katja Schenke-Layland[1,5,6]

[1] *Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany*

[2] *Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", University of Tübingen, Tübingen, Germany*

[3] *Fraunhofer IGB, Stuttgart, Germany*

[4] *Discipline of Anatomy, School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Galway, Ireland*

[5] *NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*

[6] *Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA*

Corresponding author's email: Daniel.Carvajal-Berrio@med.uni-tuebingen.de

Fluorescence lifetime imaging microscopy (FLIM) is an imaging technology that allows the time resolution of fluorophores. This extra time-dimension transforms every single fluorophore into a microenvironment probe capable of reacting to changes in its surroundings. Coupled with multiphoton microscopy, FLIM enables the contact and marker free evaluation of cells due to the autofluorescence of endogenous free/bound reduced nicotinamide adenine dinucleotide (NADH). In this work, we employed FLIM NADH for the monitoring of cellular metabolism and death in 2-dimenttional human dermal fibroblast (hDFs) and 3-dimentional EndoC-ßH3 insulin-producing pseudo islets. Induced cell death in hDFs and hypoxia-induced damages for pseudo-islets resulted in an overall increment of fluorescent lifetimes of both bound and free NADH species. Moreover, as pseudo-islets are glucose responsive, fluorescence lifetime fluctuations throughout a glucose-stimulated insulin secretion (GSIS) assay revealed the metabolic integrity differences between normoxic and hypoxic pseudo islets. Here, we propose FLIM as a tool for the evaluation of cell integrity and functionality.

Keywords

Fluorescence lifetime imaging microscopy; cell death ; cell integrity and functionality

Abstract **#851**

CAN THE CONCEPT OF FUNCTIONAL FIBROSIS BE INTENTIONALLY EXPLOITED TO YIELD IMPROVED OUTCOMES FOLLOWING VOLUMETRIC MUSCLE LOSS INJURIES?

Christopher L. Dearth[P][1,2], Connor Dolan[1,2], Sarah Franco[1,2], Naveena Janakiram[1,2], Michael Valerio[1,2], Stephen M. Goldman[1,2]

[1] *DoD-VA Extremity Trauma & Amputation Center of Excellence*

[2] *Uniformed Services University of the Health Sciences*

Corresponding author's email: connor.dolan.ctr@usuhs.edu

It is plausible that the functional improvements achieved by regenerative therapies for volumetric muscle loss (VML) to date are due, in part, to the induction of fibrotic bridge across the VML defect – a concept termed 'functional fibrosis'. However, to date, this concept has not been definitively experimentally proven. The purpose of this study was to evaluate the therapeutic potential of intentionally inducing functional fibrosis as a means to promote improved muscle function following VML. A rat tibialis anterior (TA) VML model was used with the following groups: 1) untreated (control), and 2) functional fibrosis, which was achieved by implanting a polypropylene mesh with a bolus of TGF-β — both of which are well known to induce fibrosis. At 4 and 8 weeks post-VML injury, in vivo functional analysis was performed and TA muscles were collected and analyzed for expression of Col1a1, Col3a1, and Acta2, among other analyses. As expected, functional fibrosis treatment of a VML injury resulted in increased Col1a1 and Acta2 expression relative to the untreated controls. Interestingly, at 8 weeks post injury, the functional fibrosis group had improved peak isometric force (61% increase) compared to untreated control. The findings presented herein support the concept of intentionally eliciting a super-physiologic fibrotic repair response as a means improving force transmission across a VML defect thereby improving the whole muscle functional capacity. As such, induction of functional fibrosis represents a plausible path forward for treating VML if the overall clinical goal of the treatment is focused on limb function.

Keywords

Extremity Trauma; Volumetric Muscle Loss; Wound Healing

Abstract **#852**

IMMUNOMODULATORY MATRIX-BOUND NANOVESICLES MITIGATE ACUTE AND CHRONIC PRISTANE-INDUCED RHEUMATOID ARTHRITIS.

Raphael J Crump[1,2], Catalina Pineda[1,2], George Hussey[1,2], Kelsey Hall[1,2], Hongshai Li[1], Stephen F Badylak[1,2]

[1] *University of Pittsburgh*

[2] *McGowan Institute for Regenerative Medicine*

Corresponding author's email: rjc105@pitt.edu

Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation and progressive destruction of synovial joints. Disease pathology is driven by an imbalance in the ratio of infiltrating pro-inflammatory vs. anti-inflammatory cell phenotypes, especially macrophages. Modulation of macrophage phenotype, specifically the M1-like to M2-like, pro- to anti-inflammatory transition, can be induced without immunosuppression by materials composed of extracellular matrix (ECM). This ECM-based immunomodulatory effect is thought to be mediated in part through recently identified matrix-bound nanovesicles (MBV) embedded within ECM that are released upon matrix degradation. MBV can modulate this M1-M2 transition. To examine the efficacy of MBV therapy for RA, MBV were delivered via intravenous (i.v.) or peri-articular (p.a.) routes to rats stimulated with pristane, an inducer of rat-RA. MBV therapy was compared to intra-peritoneal (i.p.) methotrexate (MTX), the standard of care. Relative to diseased animals, i.p. MTX, i.v. MBV, and p.a. MBV significantly reduced arthritis scores in both acute and chronic pristane-induced arthritis ($p<0.05$). There was no significant difference observed among i.p. MTX, i.v. MBV, and p.a. MBV in both phases ($p>0.05$). Post-mortem examination of joint tissue by histology and microCT at day 100 showed that both i.v. MBV and p.a. MBV reduced synovial inflammation, bone erosion, and the ratio of synovial M1:M2 macrophages compared to diseased animals ($p<0.05$). The results presented demonstrate that administration of MBV are equally efficacious to MTX for management of both acute and chronic pristane-induced arthritis and this effect is associated with immunomodulation of synovial macrophages, without immunosuppression.

Abstract **#853**

ESTABLISHMENT OF A 3D LUNG TUMOUR MODEL IN FIBRIN HYDROGELS WITH TRI-CULTURES

Caroline Kniebs[P][1], Anja Elisabeth Luengen[1], Daniel Guenther[1], Christian Gabriel Cornelissen[2,1], Stefan Jockenhoevel[1,3], Anja Lena Thiebes[1]

[1] Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany

[2] Clinic for Pneumology and Internal Intensive Care Medicine (Medical Clinic V), RWTH Aachen University Hospital, Aachen, Germany

[3] AMIBM-Maastricht University, Geleen, The Netherlands

Corresponding author's email: kniebs@ame.rwth-aachen.de

The importance of 3D in vitro models has increased to replace animal studies, which are often not transferable to humans. However, even these models do not completely mimic the native tumour microenvironment: In previous multicellular 3D tumour models, which represent the interaction between tumour cells and non-malignant cells, different relevant cell types were chosen, but the tumour microenvironment was represented in a simplified way regarding to tumour cell migration and the surrounding vascular network.

In this study, we focussed on developing a vascularized 3D tumour model based on fibrin gel for in vitro testing of chemotherapeutic agents.

We co-cultured HUVECs and mesenchymal stem cells acting as pericytes in fibrin gel for 14 days at 37 °C and 5 % CO2. Lung tumour cells (A549) were subsequently injected into the gel by a punch-out method and maintained in culture for another 7-14 days under normoxic and hypoxic conditions. The tri-cultures were treated with a chemotherapeutic agent and analysed by 2-photon laser scanning microscopy. Cultivation of co- and tri-cultures under hypoxic conditions promoted vascularization as shown by significantly increased amounts of vascular structures and branching points as well as increased secretion of vascular endothelial growth factor compared to normoxic-cultivated controls.

The resulting functional tumour network was reduced by the addition of a tumour drug. Our data provide the first step towards patients individualised 3D tumour models and their application as an approach to effective therapeutic in vitro testing.

Abstract **#854**

BIOCHEMICAL FUNCTIONALIZATION OF THE DENTAL ABUTMENT MATERIALS TI6AL4V AND Y-TZP THROUGH COVALENT CONJUGATION OF ECM PROTEINS AND THEIR EFFECT ON THE CELLULAR BEHAVIOR OF GINGIVAL FIBROBLASTS

Alena Lisa Palkowitz$_P$[1], Taskin Tuna[2], Shaza Bishti[2], Frederik Böke[3], Nathalie Steinke[4], Gerhard Müller-Newen[5,6], Stefan Wolfart[2], Horst Fischer[1]

[1] *Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany*

[2] *Department of Prosthodontics and Biomaterials, RWTH Aachen University Hospital, Aachen, Germany*

[3] *Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany*

[4] *Flow Cytometry Facility, Faculty of Medicine of RWTH Aachen University, Aachen, Germany*

[5] *Institute of Biochemistry and Molecular Biology, RWTH Aachen University Hospital, Aachen, Germany*

[6] *Confocal Microscopy Facility, Faculty of Medicine of RWTH Aachen University, Aachen, Germany*

Corresponding author's email: apalkowitz@ukaachen.de

For the long-term success of percutaneous components like dental implants, a functional attachment of the soft tissue to the surface of the implant abutment is of decisive importance in order to prevent the penetration of bacteria into the implant-bone interface which can trigger peri-implant diseases. Here, we describe a surface modification approach, which includes the covalent immobilization of the extracellular matrix (ECM) proteins fibronectin and laminin via a crosslinker to silanized Ti6Al4V and Y-TZP surfaces. The surface properties were evaluated by static contact angle, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). Retained function of the ECM-proteins after surface coupling was shown in a centrifugation-assay. Here, human gingival fibroblasts (HGFs) seeded onto ECM protein modified surfaces exhibited significantly higher attachment in comparison to non-functionalized controls. Confocal microscopy revealed a much higher cell area and enhanced expression of pFAK-Y397 on ECM protein coated surfaces compared to those seeded on native surfaces. Moreover, HGFs cultivated on ECM protein coated surfaces showed a significantly higher proliferation rate and integrin expression. The presented approach holds great potential to enable a stronger binding between soft tissue and implant abutment surface, which could help to prevent the penetration of bacteria and thereby reduce the risk of peri-implant diseases.

Keywords

dental implant; ECM proteins; gingival fibroblasts

Abstract **#856**

ELASTIN-LIKE-RECOMBINAMER CRYOGEL AS A PLATFORM FOR LUNG REGENERATION

Zackarias Söderlund$_P$[1], Linda Elowsson Rendin[1], Solmaz Hajizadeh[2], Emil Tykesson[1], Arturo Ibáñez-Fonseca[3], José Carlos Rodríguez-Cabello[3], Li Ye[2], Gunilla Westergren-Thorsson[1]

[1] Lung biology, Department of Experimental Medical Science, Lund University, Lund, Sweden

[2] Division Pure and Applied Biochemistry, Lund University, Lund, Sweden

[3] BIOFORGE Lab, Universidad de Valladolid, Valladolid, Spain

Corresponding author's email: Zackarias.soderlund@med.lu.se

INTRODUCTION:

Chronic obstructive pulmonary disease (COPD) is a worldwide health problem, where the tissue is gradually degraded, leading to emphysema and loss of tissue integrity [1]. Our goal is to improve regeneration of the damaged tissue by inserting a biomaterial with the ability to activate regeneration in the border zone between destroyed areas and remains of healthy tissue in COPD. Elastin-like Recombinamer (ELR) cryogel is a type of biomaterial that has proved to have excellent biocompatibility properties [2].

METHODS:

ELR is a two-part solution which are modified so that one part has an alkyne modification while the second part has an azide modification. When these two parts are mixed a covalent bond is formed resulting in a hydrogel. ELR cryogel was created by forming the hydrogel in subzero temperatures. A549 cells with GFP were seeded on top of the cryogel and grown for seven days, evaluated according to morphology and distribution in confocal microscopy.

RESULTS AND DISCUSSION:

The results show that the cells attach to the surface of the cryogel and per se to be able to migrate inward towards the center of the cryogel over time. The next step in this project is co-culturing the cryogel with primary human type 1 cells together with primary fibroblasts might lead to cells forming more alveolar like structures and the fibroblast adding native ECM to the gel.

CONCLUSION:

The data showed that an ELR-based cryogel is a promising synthetic scaffold for lung tissue engineering, mimicking the 3D environment of the extracellular matrix.

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Abstract **#857**

CAN ASC SECRETOME-LOADED DECELLULARIZED EXTRACELLULAR MATRIX (ECM) HYDROGELS AUGMENT WOUND HEALING IN A SKIN FLAP?

Linda Vriend[P][1,2,3], Joris A. van Dongen[1,4,3], Cristina P. Camargo[5], Berend van der Lei[2,6], Martin C. Harmsen[1]

[1] *University and Medical Center Groningen - Medical Biology & Pathology*

[2] *University and Medical Center Groningen - Plastic & Reconstructive Surgery*

[3] *Federal University of São Paulo (FMUSP) / InCor Heart Institute São Paulo*

[4] *University and Medical Center Utrecht - Plastic Surgery*

[5] *Federal University of São Paulo (FMUSP) - Plastic Surgery*

[6] *Bergman Clinics Hilversum*

Corresponding author's email: lindavriend@gmail.com

Introduction

Impaired wound healing is a health problem with limited treatment options. Autologous fat grafting may improve wound- and scar treatment. We hypothesized that adipose tissue-derived stromal cells' secretome and their profiency to bind to ECM underlies this regenerative effect. To test, we developed injectable ASC-secretome-loaded ECM-hydrogels to improve dermal wound healing in rats.

Methods

Decellularized pigskin (ECM) was finely powdered and mildly pepsin-digested to yield liquid pre-gels that gelated at 37°C. Human ASCs were from human lipoaspirates were used to prepare serumfree conditioned medium (ASCcme i.e. secretome). Full thickness dorsal skin flaps (3x10cm) were used to fill with ASCcme-loaded skin hydrogels. Gelation was within seconds Controls were gel or ASCcme alone or saline. At 1, 2 and 4 w, explanted wounds were assessed by H&E (general histology), Masson's Trichrome (matrix remodelling and fibrosis) and CD68 staining (macrophages).

Results

Decellularized ECM was free of cellular&DNA remains. Thin sections' (immune)histochemical analysis of excised wounds showed that during the course of wound healing, vascularization increased with angiogenesis scores highest after 2w. Collagen remodeling showed similar patterns in fiber length and angle, except for fiber width, which was significantly thinner in the ECM-hydrogel group. The presence of bare or ASCcme-loaded hydrogels did not influence wound healing nor did it cause adverse reactions at all times. Likely, the dose of ASCcme needs to be increased to accelerate wound dermal wound healing.

Conclusion

ECM-hydrogels are well-tolerated by rats in healing dermal wounds.

Keywords

ASCs; ECM-hydrogel; RegenerativeMedicine

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Abstract **#858**

USING BIOPRINTING TO INTEGRATE HIGH CELL DENSITY HYDROGELS WITH METAL IMPLANTS FOR ENHANCED OSSEOINTEGRATION

Marcin Kotlarz$_P$[1], Ana Ferreira-Duarte[1], Piergiorgio Gentile[1], Kenny Dalgarno[1]

[1] *Newcastle University*

Corresponding author's email: m.kotlarz2@newcastle.ac.uk

The surface of metallic implants serves as a powerful signalling cue for cells. Its properties play an important role in facilitating the early osseointegration by encouraging bone deposition on the surface and stabilising bone-implant interface. In this work, we demonstrated the application of a bioprinting process called Reactive Jet Impingement (ReJI) [1] to fabricate cell-laden coatings onto medical implants, with the ultimate aim of accelerating osseointegration through stimulating endogenous cells.

Natural hydrogel coatings consisting of collagen, alginate and fibrin [2] that delivered high cell densities (4 x 10^7 cells/ml) of human TERT immortalised bone marrow stromal cells were deposited onto the Ti6Al4V ELI titanium alloy implants. Three types of metal substrate with different surface roughness and morphology were used. The constructs were cultured over 21 days and assessed using Live/Dead, SEM, immunofluorescence and Alizarin Red.

Cell viability was not affected by the bioprinting process. Topographical and morphological features of the surface influenced cell organisation and cell-metal surface communication. All types of Ti6Al4V ELI implants supported the coating attachment. Cell migration and direct cell-metal implant interactions were visible from day 1. Significant calcium deposition in the area where the gel coating was printed was observed.

Morphologically robust and organised architectures were produced thanks to the drop-by-drop deposition and mixing of bio-inks at nano-litre scale using the ReJI process. High cell densities enhanced cell-biomaterial interactions at the hydrogel/metal surface interface and the coating supported the formation of a layer of functional bone-like tissue on the Ti6Al4V ELI implants.

Keywords

tissue-engineered implants; hydrogel coating; cell-material interactions

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Abstract **#859**

SCAFFOLD-BASED DELIVERY OF PSDF-1A TO A CO-CULTURE SYSTEM OF MESENCHYMAL STEM CELLS AND ENDOTHELIAL PROGENITOR CELLS FOR BONE REPAIR

Rachael Power[1,2], Lia Blokpoel Ferreras[1,2], Arlyng Gonzalez Vasquez[1,2], Brenton Cavanagh[3], James E Dixon[4], Kevin M Shakesheff[4], Caroline M Curtin[1,2], Fergal J O'Brien[1,2]

[1] Tissue Engineering Research Group, Royal College of Surgeons in Ireland

[2] Advanced Materials and Bioengineering Research Centre (AMBER), Trinity Centre for Bioengineering and Trinity Biomedical Sciences Institute

[3] Cellular and Molecular Imaging Core, Royal College of Surgeons in Ireland

[4] School of Pharmacy, University of Nottingham, UK

Corresponding author's email: rachaelnpower@rcsi.com

Following a challenging history of viral gene delivery vectors, non-viral vectors have gained popularity in regenerative medicine. Our lab has pioneered gene-activated scaffolds for bone repair (1-3), most recently using the glycosaminoglycan-enhanced transduction (GET) peptide (4). A modified peptide (GET*) facilitates enhanced endosomal escape, thereby increasing transfection efficiency. The aim of this study was to optimise GET*pDNA nanoparticles for collagen-nanohydroxyapatite (coll-nHA) scaffold-based delivery (5) of a plasmid encoding stromal derived factor 1α plasmid (pSDF-1α), a chemokine which promotes bone repair by increasing angiogenesis (6). pSDF-1α plasmids were delivered to a co-culture system of mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs), cell types which promote bone regeneration (7).

GET* nanoparticles were characterized at multiple N/P ratios by transfecting MSCs and EPCs with reporter plasmids in 2D and via two different scaffold compositions (coll-nHA 100% and 200% wt. scaffolds). pSDF-1α was then delivered to MSCs, EPCs, and co-cultures using the optimal GET* nanoparticles incorporated into coll-nHA scaffolds before quantifying the therapeutic effect.

GET* N/P 8 nanoparticles demonstrated the highest transfection efficiency in 2D monolayer and on both coll-nHA scaffold compositions. Delivery of pSDF-1α using this optimal gene-activated scaffold platform resulted in significantly higher calcium production in a co-culture of MSCs to EPCs compared to untransfected controls.

In conclusion, GET* N/P 8 nanoparticles demonstrated the highest transfection efficiency compared with other groups, in 2D monolayer and via coll-nHA scaffolds. pSDF-1α delivered to an MSC/EPC co-culture system using this platform showed promise in vitro, suggesting that the pSDF-1α is enhancing osteogenesis.

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Abstract **#860**

A QUANTITATIVE REVIEW OF THE RELATIONSHIP BETWEEN SI RELEASED FROM BIOACTIVE GLASSES AND THEIR CELLULAR INTERACTIONS.

Joel Turner$_P$[1,2], Arkhash Nandakumar[1], Julian Jones[2], Gavin Jell[1]

[1] *University College London*

[2] *Imperial College London*

Corresponding author's email: joel.turner.17@ucl.ac.uk

Despite 50 years of silicate bioactive glasses (SBG) research (with over 100 different SBGs formulations and in excess of 500 papers) there remains a lack of understanding of how soluble silica species (Si) released from the SBGs influences cellular responses. Using a systematic approach, this paper quantitatively compares the in-vitro responses of cells to BG dissolution products reported in literature (90 publications that quantify silicate ion concentrations [Si]). Both [Si] and changes to cellular behaviour (percentage difference relative to a control) were then collected from each paper.

The [Si] that most papers report desirable cellular responses (within 1-week culture) is between 1 & 40ppm. Si concentrations above 40ppm are almost 4 times more likely to cause unfavourable cellular responses. Cell-type and species specific differences in cellular responses were also found, where for example, an increase in proliferation is observed in response to [Si] in cells derived from human species ($P<0.001$), but not other species. There was, however, no statistical difference between the [Si] that caused altered expression of ALP, osteocalcin, collagen, VEGF or in vitro mineralisation in the reported literature, suggesting Si may not influence their expression or that insufficient evidence is available.

This review has, for the first time, quantitatively compared the cellular responses to SBGs from the literature . Whilst evidence is presented for the range of [Si] that cause positive cellular responses (<40ppm), this review demonstrates the need for greater standardisation of in-vitro methodological approaches.

Keywords

Silica; Bioactive-glass; In-vitro

Abstract **#861**

ENGINEERING 3D MICROENVIRONMENT TO TEST KERATOCYTES BEHAVIOR

Rebeca Rivero[1], Antonio Feliciano$_P$[1], Stefan Giselbrecht Giselbrecht[2], Lorenzo Moroni[1], Matthew Baker[1]

[1] Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University

[2] Departament of Instructive Biomaterial Engineering, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University

Corresponding author's email: r.rivero@maastrichtuniversity.nl

Corneal disease is the fourth cause of blindness. To advance knowledge towards a tissue-engineering approach for corneal regeneration, information about biomaterials-tissue/cell interactions is needed. To develop such models, it is crucial to mimic the cornea in-vivo microenvironment.

The cornea is the outermost transparent part of the eye, and consists of five layers. Stroma is the thickest of the layers, composed of well-organized collagen fibrils and quiescent keratocytes. Keratocytes during in-vitro culture easily undifferentiate; however, most of these studies are conducted on 2D monolayers.1

To engineer a functional corneal in-vitro model, it is imperative to better understand the cornea structure and the effects of a variety of signals seen in-vivo, such as mechanical properties. 2

Here, we have formulated hydrogels based on UV-cross-linked alginate3 along with supramolecular or collagen based reinforcing networks. This has allowed us to control the processability, mechanical properties, and optical clarity of hydrogels to study keratocytes behavior in hydrogels with varying stiffness and shapes.

Hydrogels mechanical properties are tunable by varying the concentration, length, and structure of the cross-linkers, as well as the composition of the final gel.

The hydrogels obtained showed a transparency of ~97% and a refractive index of 1.333 (human cornea: 1.376).4 The effects of the hydrogel mechanical properties on the keratocytes is being studied by the expression of alpha-smooth muscle actin and aldehyde dehydrogenase-1A1.

Understanding the effects of the mechanical properties of the material on the keratocytes behavior is crucial to the development of a tissue-engineered cornea and biomaterial implants for the cornea.

Keywords

Hydrogels; Keratocytes

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Abstract **#862**

FUSED DEPOSITION MODELLING OF COMPOSITE BLENDS FOR BONE TISSUE ENGINEERING SCAFFOLDS: TOWARDS THE PERSONALIZED TREATMENT OF OSTEOPOROTIC FRACTURES

Amedeo Franco Bonatti[P][1], Gabriele Maria Fortunato[1], Aurora De Acutis[1], Carmelo De Maria[1], Paulo Quadros[2], Kenneth Dalgarno[3], Raasti Naseem[3], Maria Chatzinikolaidou[4,5], Rafaela-Maria Kavasi[4], Georgia-Ioanna Kontogianni[4,5], Giorgia Montalbano[6], Sonia Fiorilli[6], Chiara Vitale Brovarone[6], Giovanni Vozzi[1]

[1] *Research Center E. Piaggio and Dpt. of Information Engineering, University of Pisa, Pisa, Italy*

[2] *FLUIDINOVA, S.A., Maia, Portugal*

[3] *School of Engineering, Newcastle University, Newcastle upon Tyne, United Kingdom*

[4] *Foundation for Research and Technology Hellas (FO.R.T.H)-IESL, Heraklion, Greece*

[5] *Department of Materials Science and Technology, University of Crete, Heraklion, Greece*

[6] *Department of Applied Science and Technology, Politecnico di Torino, Turin, Italy*

Corresponding author's email: bonattiamedeo@gmail.com

Osteoporosis is a bone pathology with a high economic and social burden, and an increasing incidence due to demographic ageing [1]. GIOTTO project aims to target specific osteoporotic fractures using smart, innovative and customized devices by leveraging advanced know-how in biofabrication technologies [2]. Blends of poly-L-lactic acid (PLLA), polycaprolactone (PCL) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (90:5:5%wt), PLLA/PCL/PHBV + 5%wt of nano-hydroxyapatite (nHA) and PLLA/PCL/PHBV + 5%wt of strontium-substituted-nHA were fabricated into 1.75 mm diameter filaments. Degradation kinetics, mechanical and rheological properties were optimized. Rheological assessment showed a shear thinning behavior in the Fused Deposition Modelling (FDM) temperature range and confirmed the inorganic phases homogenous dispersion. The filaments were processed using a FDM-printer. Preliminary extrusion and printing tests were performed to optimize the process parameters, which were thus employed to produce cylindrical scaffolds for biological testing. The scaffolds were cultured in contact with pre-osteoblastic and bone marrow-derived mesenchymal stem cells. Cytotoxicity and morphology were evaluated by a colorimetric viability assay and scanning electron microscopy, respectively. The osteogenic potential was screened using alkaline phosphatase (ALP) activity measurement, alizarin red staining and sirius red staining. All materials demonstrated high cell viability, excellent adhesion and morphology. The addition of both inorganic phases indicated a significantly higher ALP activity and calcium mineralization when compared to the control. Finally, all blends showed an increasing collagen secretion. These results indicate the high osteogenic potential of the developed materials.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 814410.

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Abstract **#863**

DEVELOPMENT AND TESTING OF A NOVEL, IMMORTALIZED, OFF-THE-SHELF CELL LINE FOR BONE HEALING

Rodolfo E. De la Vega$_P$[1,2], Michael J. Coenen[1], Gresin Hawse[1], Christopher H. Evans[1]

[1] Musculoskeletal Gene Therapy Research Laboratory, Rehabilitation Medicine Research Center, Mayo Clinic, Rochester, MN, USA

[2] Department cBITE and Department IBE, MERLN – Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

Corresponding author's email: delavega.rodolfo@mayo.edu

Bone morphogenetic protein-2 (BMP-2) is the most potent, clinically-approved, osteogenic cytokine. Yet, it has limited approved use, requires supraphysiological doses, and side effects are a concern. Gene transfer has been suggested to improve and prolong its delivery. Ex vivo gene transfer holds advantages as a therapy1, but the use of autologous cells is limited by the high-cost associated with harvesting and/or tissue culture expansion prior to re-implantation. This project aimed to overcome this limitation by generating a non-osteogenic, stably transduced, immortalized cell line that would serve as an off-the-shelf allogeneic product for bone healing.

HEK293 cells were lentivirally transduced to express BMP-2 constitutively. Clones were selected, expanded, characterized and frozen at 1x106 cells/vial. BMP-2 production was measured by ELISA. Individual vials were thawed and cells encapsulated in fibrin prior to surgical implantation in a rat, 5-mm, femoral, bone defect model. Defect bridging was monitored via weekly radiographs. Empty defects in this model fail to bridge the defect. FK506 was used to prevent xenograft rejection.

Clone CL1K was selected and BMP-2 expression confirmed for 50 passages. After 6 weeks, 5 rats presented with bicortical bridging, 2 with unicortical and 3 without bridging.

The ability of the BMP-2 expressing CL1K cells to bridge large bone defects while lacking intrinsic osteogenic potential is a novel approach to bone healing by gene transfer. The prospect of an off-the-shelf cell line for bone healing is a promising clinical solution. Future work is needed to optimize the conditions needed for this approach to be reliably successful.

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Abstract **#864**

3D BIOPRINTING WITH REJUVENATED CELLS AND OA PROTECTIVE NANO-INK IN SHAPE OF AN OA LESION

Stina Simonsson[P][1], Bruno Sarmento[2,3], Catarina Pereira Leite[2,3], Meriem Lamghari[2,3], Carl Lindahl[1]

[1] *University of Gothenburg, Sweden*

[2] *I3S – Instituto de Inovação e Investigação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-125 Porto, Portugal*

[3] *INEB – Instituto de Engenharia Biomédica, Rua Alfredo Allen, 208, 4200-125 Porto, Portugal*

Corresponding author's email: stina.simonsson@gu.se

Objective: Cartilage injuries can lead to osteoarthritis (OA) that cause cartilage loss, bone remodelling and osteophyte development and remain a therapeutic challenge. Medical engineering by 3D bioprinting with cells and therapeutic ink has potential to reconstruct patient-specific features that for example match an articular joint lesion.

Design: Here a human osteoarthritic (OA) tibial plateau with a cartilage defect was retrieved after total knee arthroplasty and scanned using following clinical imaging techniques; (I) computed tomography (CT), (II) magnetic resonance imaging (MRI) or (III) a three-dimensional (3D) scanner. Ink for 3D bioprinting with nano-carriers with a potential OA protective capacity was developed.

Results: Highest resolution was obtained using the 3D scanner and only the 3D scanner was able to detect the actual OA defect area. Human chondrocytes or micro cartilage tissue, generated from induced pluripotent stem cells (iPSCs), included in 3D bioprinted constructs produced extracellular matrix (ECM) and formed cartilage tissue fragments after two weeks of differentiation. High levels of a mature splice version of collagen type II (Col IIA type B), characteristic of native articular cartilage, and aggrecan (ACAN) were detected. 3D bioprinted micro tissue embedded with nanomaterials with enhanced cartilage repair or capable of mitigating illicit biological responses were safe in mice immune deficiency model.

Conclusion: Our results establish that articular cartilage similar to its native form can be made in shape of patient-specific 3D lesion. Further, nanomaterials loaded with OA combating molecules can have direct implications in personalised medicine and provides an attractive strategy for future treatments of OA.

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Abstract **#865**

VASCULARIZED MICRO-PHYSIOLOGICAL MODELS FOR STUDYING MULTICELLULAR-VASCULAR INTERACTIONS AND NANOCARRIER DRUG TRANSPORT THROUGH MICROFLUIDIC TECHNOLOGIES

Marco Campisi[1,2], Tatsuya Osaki[3,4], Sarah Shelton[2,5], Shriram Sundararaman[2,6], Sharon Lee[7,8], Clara Mattu[9], Giulia Adriani[8], Claudia Voena[10], Ines Mota[10,11], Enrico Patrucco[10], Shunsuke Kitajima[2], Roberto Chiarle[10,11], David Barbie[2], Roger Kamm[4,5], Valeria Chiono[9]

[1] Dept. of Mechanical and Aerospace engineering, Politecnico di Torino, Turin, Italy

[2] Dept. of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, US

[3] Institute of Industrial Science, University of Tokyo, Japan

[4] Dept. of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, US

[5] Dept. of Biological Engineering, Massachusetts Institute of Technology, Cambridge, US

[6] University of Virginia School of Medicine, Charlottesville, US

[7] Singapore-MIT Alliance for Research and Technology (SMART), Singapore, Singapore

*[8] Singapore Immunology Network (SIgN) Agency for Science, Technology and Research (A*STAR) Singapore, Singapore*

[9] Dept. of Mechanical and Aerospace engineering, Politecnico di Torino, Turin, Italy

[10] Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Turin, Italy

[11] Dept. of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, US

Corresponding author's email: marco.campisi@polito.it

The recent application of microfluidic technologies in bioengineering has driven considerable progress in the design of 3D micro-tissues on chips, which offer the possibility to improve preclinical experimentation respect to 2D culture systems, in agreement with the 3Rs principle. Commonly referred to as microphysiological systems, such biomimetic in vitro models replicate the in vivo tissue-specific microenvironments making use of human induced pluripotent stem cells (iPS) or primary cells embedded in extracellular matrix (ECM)-like hydrogels. Here, we designed three biologically-inspired 3D microphysiological models of multicellular-vascular interactions in microfluidic devices: human Blood-Brain barrier (BBB)[1], KRAS/LKB1 lung tumor and ALK-positive Anaplastic Large Cell Lymphoma (ALCL) model. These models have in common advanced perfusable microvasculature, obtained through self-assembled vasculogenesis or 3D macrovessel culture and supported by cell-cell dynamic contact interactions and continuous secretion of signaling factors. The BBB model, based on iPS-derived cells, showed increased maturation toward BBB-like structures with vascular permeability lower than conventional in vitro systems, and it was used to test transport of innovative carriers, such as polymer nanoparticles[2]. The lung tumor-microvascular model was critical to understand the biology of immune cell recruitment and exclusion in immunotherapy-resistant lung tumor microenvironment. The ALCL model was exploited to unveil a molecular mechanism of tumor drug resistance. These advanced and physiologically-relevant models have the potential to accelerate drug discovery by reliable prediction of therapeutic vulnerabilities and biotransport studies of drugs across barriers, thereby improving the understanding of several currently incurable diseases.

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Abstract **#866**

PREPARATION OF DECELLULARIZED DERMIS USING SUBCRITICAL DME

Naoko Nakamura$_P$[1], Kota Tanabe[1], Hiroko Fukuhara[1], Masatoshi Shimotomai[1], Satoshi Shinohara[2], Shogo Suzuki[2], Tsuyoshi Kimura[3], Akio Kishida[3]

[1] *Shibaura Institute of Technology*

[2] *RICOH*

[3] *Tokyo Medical and Dental University*

Corresponding author's email: naoko@shibaura-it.ac.jp

In recent years, decellularized tissues have been used as biomaterials for transplantation. Although many decellularized tissues that have been clinically applied are prepared using a surfactant, we then have developed a novel decellularization method using subcritical dimethyl ether (DME) instead of the surfactant. Perfusing the subcritical DME are usually used for lipid extraction. Therefore perfusing the subcritical DME would be able to destroy and remove the cell membrane composed of phospholipids. Since DME vaporized at room temperature and pressure, it is expected that it would not remain in the decellularized tissues. We have here prepared decellularized dermis by the subcritical DME perfusion and investigated the amount of residual DNA and the maintenance of tissue structure. The dermis was prepared by removing the epidermis and the fat layer from the porcine skin and trimmed into a disk shape with a diameter of 8 mm. The samples were placed in the extraction container, and perfused in a fixed direction for 5 hours with liquefied DME by applying a pressure of 0.7 MPa. The samples were then shaken in DNase solution to decompose DNA and remove cellular components. Quantitative DNA evaluation and histological evaluation were performed. It was revealed that our method using subcritical DME perfusion and DNA enzymatic degradation could prepare the decellularized dermis as same as the method using surfactant. It was suggested that the possibility of developing a novel decellularization method using subcritical DME.

Abstract **#867**

HETEROGENOUS RIBOSOMES IN HUMAN DERMAL FIBROBLAST SENESCENCE

Markus Schosserer$_P$[1,2], Fabian Nagelreiter[1], Guohuan Yang[1,3], Lisa Liendl[1], Clemens Heissenberger[1], Yulia Gonskikh[4], Norbert Polacek[4], Martin Kos[3]

[1] *University of Natural Resources and Life Sciences, Vienna, Institute of Molecular Biotechnology, Austria*

[2] *Austrian Cluster for Tissue Regeneration, Austria*

[3] *Biochemistry Center (BZH), Heidelberg University, Germany*

[4] *University of Bern, Switzerland*

Corresponding author's email: markus.schosserer@boku.ac.at

For a long time, the ribosome has been regarded as a static machine and all ribosomes within a cell were assumed to be identical. This simplified view has been challenged in recent years, when the existence of heterogeneous ribosome populations was reported. Heterogeneous ribosomes may differ in rRNA, ribosomal protein composition or differential modifications of ribosomal proteins or rRNA. However, the functional consequences of these structural specializations are still poorly understood.

Presence of senescent cells in vivo is associated with several age-related changes. Using human dermal fibroblasts (HDFs) as a model, we found increased global protein synthesis and nucleolar size in senescent cells. As the underlying mechanisms of these effects are still unknown, we explored if specific methylations of the ribose backbone contribute to the senescent phenotype.

We quantified methylation levels across all positions of rRNA. Thereby, we identified several differentially methylated sites in senescent compared to proliferating and quiescent cells. We identified several positions to be hypo- or hyper-methylated in quiescent and senescent cells in comparison to proliferating cells. Methylation levels were highly correlated with expression of snoRNAs mediating these modifications. Currently we are testing functional consequences of depletion of these differently regulated snoRNA on cellular physiology and ribosome function.

Taken together, our data suggest that even subtle modifications of the ribosomal RNA might have profound and very specific effects on cellular physiology and contribute to the specialization of ribosomes.

Abstract **#868**

PLACENTAL STEM CELLS EFFICACY IN A PRECLINICAL KIDNEY INJURY MODEL

Agnė Gryguc$_P$[1], Justinas Mačiulaitis[2], Lukas Mickevičius[3], Arvydas Laurinavičius[4], Neringa Sutkevičienė[5], Inga Arūnė Bumblytė[1], Romaldas Mačiulaitis[1,2]

[1] Department of Nephrology, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania

[2] Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Kaunas Lithuania

[3] Department of Urology, Hospital of Lithuanian University of Health Sciences, Kaunas Lithuania

[4] National Center of Pathology, Affiliate of Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania

[5] Large Animal Clinic, Veterinary academy, Lithuanian University of Health Sciences, Kaunas Lithuania

Corresponding author's email: agneihana@hotmail.com

Introduction

Acute kidney injury is affecting about 20% of surgically treated patients.1,2,3

Placental mesenchymal stem cells (PSCs) have been shown to possess anti-inflammatory and immunomodulatory properties.4

Methods

After the removal of maternal decidua basalis, placental amniotic and chorionic cells have been isolated, cultivated for four weeks and characterized by yield, viability, flow cytometry and potency in vitro.

Wistar rats underwent kidneys ischemia-reperfusion injury. Index group received 3x105 PSCs and Control group – vehicle in the corticomedullar region of each kidney.

Survival was analyzed. Urine and blood serum samples were collected on days 0, 3 and 7. Histological kidney analysis – days 3 and 7.

Results

PSCs had a consistent yield, viability, MSCs markers expression and suppressed proliferation of T cells in a numerical fashion. Survival rate improved – 100% (Index) vs. 80% (Control).

Creatinine clearance in Index group was comparable to healthy control on day 7. Normal serum creatinine, urea and Na+ was retained in Index group up to day 7.

Extensive acute tubular necrosis (ATN) with broad coagulative tubular epithelial necrosis was evident after 3 days in the Control. A clear tendency for a lower ATN area, loss of brush border and tubular dilatation and ATN without significant coagulative necrosis was evident in Index group up to day 7.

Conclusion

PSCs have the potential to prevent initial kidney fibrosis cascade through ameliorating initial kidney damage. A superior 100% survival rate of treated animal group exhibits the potential of characterized PSCs to be used in a larger scale preclinical study.

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Abstract **#869**

CELL-MATERIAL INTERACTION IN BIOSYNTHETIC HYDROGELS FOR BIOMIMETIC NEURAL INTERFACES

Martina Genta$_P$[1], Catalina Vallejo-Giraldo[1], Josef Goding[1], Rylie Green[1]

[1] *Imperial College London*

Corresponding author's email: m.genta18@imperial.ac.uk

Neural interfaces in bionic devices are dominated by the foreign body response upon implantation. Tissue engineering of the neural interface by addition of cell-laden hydrogel coatings to bionic devices has the potential to improve device-tissue integration and device efficacy. A significant challenge in this space is the development of a suitable material platform able to support neuronal viability and function. This study evaluates the incorporation of different percentages of gelatin in poly(vinyl alcohol) - norbornene (PVA-NB-Gel) hydrogels to create a construct capable of providing topographical and biochemical guidance cues to encapsulated neural cultures. The mass loss and the Young's moduli of the PVA-NB-Gel hydrogels showed degradation of the material over a period of time of 28 days, with the Young's moduli ranging from 30 to 0.5 kPa and the rate of degradation being dependent on gelatin content. Cytocompatibility of PVA-NB-Gel hydrogels was tested by encapsulating primary rat embryonic ventral mesencephalic cells. The growth and migration of astrocytes, cells responsible for neural development in a mixed cell population, were confirmed assessing the YAP expression and MMP-2 production. The study of these cellular factors, coupled with standard morphological immunostaining, provided essential insights towards the understanding of cell-material interaction in complex 3D cultures. While additional topographical cues are necessary to create fully functional 3D neuronal networks, this work demonstrated the importance of studying cell behaviour at the molecular level to tailor both the degradation profile and the biological components of a biosynthetic hydrogel for living bionic devices.

Abstract **#871**

AN INJECTABLE ALGINATE HYDROGEL FOR RESTORING SALIVARY GLAND FUNCTION AFTER RADIATION

Chelsea S Bahney$_P$[1,2], Lionel Berthoin[1], Shirin Hooshfar[1], Oju Jeon[3], Lionel Berthoid[1], Jiun-Chiun Chang[1], Seayar Mohabbat[1], Eliza Gaylord[1], Nicole Ehhrhart[4], Eben Alsberg[3], Sarah M Knox[1]

[1] *University of California, San Francisco (UCSF)*

[2] *Steadman Philippon Research Institute*

[3] *University of Chicago, Illinois (UCI)*

[4] *Colorado State University (CSU)*

Corresponding author's email: cbahney@sprivail.org

Xerostomia, or dry mouth, affects >8 million people in the U.S and occurs as a result of salivary dysfunction due to autoimmune disease, medications, smoking, aging and radiation therapy for head and neck cancer. Absence of saliva severely compromises the oral health and quality of life of patients. There are no viable long-term treatments for salivary dysfunction, driving a need for new, translatable solutions. We have devised a promising new therapy for improving salivary gland function that is based on stem cell-mediated regeneration via local delivery of an acetylcholine mimetic, cevimeline, encapsulated in an injectable alginate hydrogel. Using a combination of in vitro and in vivo assays, along with a novel mass spectrometric quantification method, we have defined the chemistry of a biodegradable oxidized alginate that enables linear distribution across the salivary gland and local release of the drug. We show that local in vivo delivery of the cevimeline-alginate hydrogel to murine salivary glands is efficacious in promoting stem cell proliferation and long-term restoration of saliva production. This study provides the first in vivo evidence that stem cells can be stimulated to re-enter the cell cycle after radiation-induced damage. In effort to move these small animal findings closer to human studies, we have developed a novel canine survival model to test safety and efficacy of our bioactive hydrogel. Together, these studies represent the first use of an injectable hydrogel for alleviating xerostomia and suggest this neurogenic-alginate hydrogel could translate to a novel system for restoring salivary function after radiation-induced damage.

Abstract **#872**

INNOVATIVE BIOINK FROM COLLAGEN AND HYALURONIC ACID WITH TUNABLE RHEOLOGICAL AND BIOLOGICAL PROPERTIES FOR CARDIOVASCULAR 3D BIOPRINTING

Sara Palladino[P][1,2], Andrea Schwab[3], Matteo D'Este[3], Francesco Copes[1], Gabriele Candiani[2], Diego Mantovani[1]

[1] Laboratory for Biomaterials and Bioengineering, CRC-I, Department of Min-Met-Materials Eng., & University Hospital Research Center, Regenerative Medicine, Laval University, Quebec City, Canada

[2] GenT Lab, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Polytechnic of Milano, Italy

[3] AO Research Institute Davos, Davos Platz, Switzerland

Corresponding author's email: sara.palladino.1@ulaval.ca

Bioprinting represents an innovative approach for the production of cellularized constructs with controlled and physiologically relevant properties. For cardiovascular regenerative medicine, microextrusion-based bioprinting is the prevalent choice. The formulation of application-specific bioinks represents a significant challenge in this field. Extrudability, shape retention and biocompatibility are essential bioink requirements, combined to adequate mechanical and rheological properties. Collagen is recognized as the gold standard, thanks to its excellent biological support for cardiovascular cells (1). However, its use as bioink is hampered by low mechanical properties and long gelation time. To overcome these limitations, in this study, collagen type I was combined with enzymatically crosslinked hyaluronic acid (HA) to formulate a bioink with tunable mechanical strength and gelation time (2) for engineering vascular constructs. Although HA use as bioink has already been proven to be suitable for tissue engineering, the co-formulation with collagen is innovative and still to be investigated.

The feasibility of this strategy has been investigated through rheological and biological characterizations, by varying horseradish peroxidase (HRP) and hydrogen peroxide (H2O2) content as mediators of HA enzymatic crosslinking. Amplitude sweep test showed the direct correlation between the HRP and H2O2 concentrations and the storage and loss moduli of the hydrogel. Preliminary viability tests were performed on vascular cell types, all showing similar trends, where the presence of collagen increases cell attachment and viability compared to bare HA.

The formulation here introduced is a promising bioink for biofabrication of collagen-based cardiovascular constructs in microextrusion bioprinting. Further optimization and printability characterization are ongoing.

Keywords

Collagen; Hyaluronic acid; Bioprinting

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Abstract **#874**

MICROENGINEERED VILLUS-LIKE PEGDA HYDROGELS UNDER SPATIO-BIOCHEMICAL GRADIENTS FOR PRIMARY INTESTINAL EPITHELIUM IN VITRO MODEL

Aina Abad$_P$[1], Gizem Altay[1], Emilio Gualda[2], María García-Díaz[1], Núria Torras[1], Jordi Folch[1], Sébastien Tosi[3], Vanesa Férnandez[1], Eduard Batlle[4], Pablo Loza[2], Elena Martínez[1,5,6]

[1] *Biomimetic systems for cell engineering, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain*

[2] *SLN Research Facility, The Institute of Photonic Sciences (ICFO), Barcelona, Spain*

[3] *Advanced Digital Microscopy (ADM), Institute For Research In Biomedicine (IRB), Barcelona, Spain*

[4] *Colorectal Cancer Laboratory, Institute For Research In Biomedicine (IRB), Barcelona, Spain*

[5] *Electronics and Biomedical engineering, University of Barcelona (UB), Barcelona, Spain*

[6] *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain*

Corresponding author's email: aabad@ibecbarcelona.eu

The intestinal epithelium is formed by villi and crypts. Intestinal stem cells (ISCs) located at the crypt base divide giving rise to proliferative cells that migrate up along the villi while differentiating, ultimately dying at the tips of the villi. This homeostasis is tightly controlled by biomolecular gradients of EGF, Wnt and BMP signaling pathways along the crypt-villus axis1. Intestinal organoids, despite including many physiologically relevant features, are not valid cultures when access to the lumen is required. Here we present a culture platform that overcomes this limitation while comprising all key features of the intestinal epithelium: 3D architecture, proliferative and differentiated cell domains, and gradients of ISCs niche biomolecules.

Employing a simple photolithographic technique2, we fabricated poly(ethylene) glycol diacrylate (PEGDA) 3D villus-like scaffolds. We developed in silico models to simulate gradients of ISCs niche biomolecules, we created them through the hydrogels by free diffusion and we characterized them by Light-sheet fluorescence microscopy.

Organoid-derived intestinal epithelial cells covered the whole scaffold surface. The gradients profile and composition, constant over time, impacted on cell behavior by modifying the proportion and positioning of the different intestinal epithelial cell types along the vertical axis of our scaffold, faithfully recreating in vivo cell compartmentalization.

We have developed an apically accessible and 3D in vitro intestinal epithelial model, which bears biomolecular ISC niche gradients and all relevant epithelial cell types. Therefore, we believe our model can be employed in many applications, particularly in the study of intestinal epithelium biology in physiological and pathological conditions.

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Abstract **#875**

BIOPRINTING AS A METHOD TO INTEGRATE HYDROGELS WITH FIBROUS MESHES FOR THE DEVELOPMENT OF CELL-LADEN CONSTRUCTS IN WOUND HEALING

Marcin Kotlarz$_P$[1], Ana Ferreira-Duarte[1], Piergiorgio Gentile[1], Stephen Russell[2], Kenny Dalgarno[1]

[1] *Newcastle University*

[2] *University of Leeds*

Corresponding author's email: m.kotlarz2@newcastle.ac.uk

Hydrogels due to their favourable properties are attractive materials for bioprinting application [1]. However, as soft materials, they often lack sufficient mechanical properties [2]. This work aimed to investigate the feasibility of the Reactive Jet Impingement bioprinting system (ReJI) as a method to integrate a hydrogel with a fibrous mesh substrate in order to improve mechanical properties and handleability of the scaffold for wound healing applications.

The ReJI system allowed for mid-air processing of low viscosity bio-inks that upon the meet, react to form a hydrogel3. The simultaneous, drop by drop bio-ink deposition brought an opportunity to print onto different surface types. Dry-laid needle-punched fibrous mesh was used as an exemplar substrate. Cell-laden hydrogel/mesh constructs were assessed using Live/Dead assay, immunofluorescence, SEM and histological analysis.

Two cell densities of neonatal human dermal fibroblasts were embedded in collagen-alginate-fibrin hydrogels and bioprinted using the ReJI system onto calcium alginate meshes maintaining high cell viability. Hydrogels supported interactions with the mesh by infiltrating into the substrate. Fibroblasts quickly migrated outside hydrogels, organising around mesh fibres and producing ECM. It was shown that the application of ReJI is an effective method for the integration of high cell density hydrogel with a fibrous substrate that could serve as a construct for wound healing applications.

Keywords

composite scaffold; bioprinitng; cell-laden construct

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Abstract **#876**

REGULATION NETWORK MODELLING TO SIMULATE CHONDROCYTE ACTIVITY AND MAP RELEVANT INFLAMMATORY MEDIATORS IN OSTEOARTHRITIS

Maria Segarra-QueraltP[1], Michael Neidlin[2], Laura Tio[3], Miguel Angel González-Ballester[4], Gemma Piella[1], Jérôme Noailly[1]

[1] *BCN MedTech, Universidad Pompeu Fabra*

[2] *Department of Mechanical Engineering, National Technical University of Athens*

[3] *IMIM,Spain*

[4] *ICREA,Spain*

Corresponding author's email: maria.segarra@upf.edu

Pharmacological treatments for osteoarthritis (OA) are based on painkillers, and none can stop OA progression because mechanisms that control pathophysiology are not well understood[1]. However, overstimulation of catabolic events of cartilage chondrocytes (CC) compared to the biosynthetic activity is common[2]. To enable mechanistic explorations of key molecular players, we propose a network-based-model that incorporates the action of cytokines at chondrocyte level. The interactome consisted in a set of nodes, related to itself through a specific topology of activating and inhibiting links. It was mathematically translated into a semi-quantitative model through a system of differential equations that converges to steady stable states[3]. The model was calibrated against experimental data by minimizing the mean absolute difference (MAD) between predicted and measured molecules through a genetic algorithm. Independent validation showed that the optimized model reduced MAD to 2.4%, against 34.4% for the non-optimized. Qualitative evaluation against literature knowledge revealed that the optimized network could reproduce OA reported molecular outcomes with 87% of accuracy. Our model could switch between a healthy and an OA state given the appropriate perturbations by local cytokines. Besides, an OA treatment based on anti-inflammatory cytokines was reproduced. Results were consistent with the reported study[4]: matrix degradation and pain mediators were reduced, but cartilage matrix was not restored, highlighting the challenge to reverse OA. Therefore, a high-level CC regulatory network that maps measurable cytokines was successfully developed. Guided network enrichment and topology optimization define a viable strategy to achieve a predictive cell regulation models, to explore mechanistically anti-degenerative therapies.Acknowledgments:HOLOA-DPI2016-80283-C2-1/2-R,RYC-2015-18888)+CatalanGovernment-FI.

Keywords

genetic algorithm; cytokine; chondrocyte

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Abstract **#877**

MICROPHYSIOLOGICAL SYSTEM TO ELUCIDATE THE ROLE OF CALCIUM IN STIMULATING VASCULOGENESIS

Adrián López Canosa[P][1,2,3], Elisabeth Engel López[1,2,4], Oscar Castaño Linares[1,2,3]

[1] Biomaterials for Regenerative Therapies, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Baldiri Reixac 10-12, 08028 Barcelona (Spain)

[2] CIBER in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 28029 Madrid (Spain)

[3] Department of Electronics and Biomedical Engineering, University of Barcelona, C/ Martí i Franquès 1, 08028 Barcelona (Spain)

[4] Department of Materials Science and Metallurgical Engineering (EEBE), Polytechnic University of Catalonia (UPC), 08019 Barcelona (Spain)

Corresponding author's email: alopezc@ibecbarcelona.eu

Over the last decades, most of the strategies to improve the vascularization of biomaterials based on calcium phosphates (CaPs) are based on the incorporation of well-known proangiogenic agents, such as growth factors, despite their high cost and the complexity of delivering safe and effective doses [1]. A promising alternative is the use of inorganic elements that naturally occur within the body, namely metallic ions [2]. It has been shown that dissolution products of CaPs are able to induce vascularization [3,4], although the particular mechanism by which calcium stimulates this process is not very well understood, mainly due to the lack of suitable in vitro and in vivo models.

In this work, we present a microphysiological system (MPS) to study the role of calcium in neovascularization. The bone-healing microenvironment was mimicked by 3D-culturing bone marrow rat mesenchymal stem cells (BM-rMSC) and rat endothelial progenitor cells (rEPC) either in mono or co-culture conditions. Migration assays were performed in our proposed system, showing that calcium-enriched media (10 mM) is only able to elicit a strong migratory response on endothelial progenitor cells when they are in co-culture conditions. We also show that calcium exerts a potent chemotactic effect on BM-rMSC and induces an increase in the osteopontin (OPN) secretion, a protein involved in chemotaxis and immune regulation [5]. Therefore, we propose a novel mechanism by which calcium can stimulate endothelial progenitor cell recruitment and subsequent vascularization and open up new possibilities to test calcium-releasing biomaterials using MPS.

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Abstract **#878**

COMPARING THE PERIPHERAL NERVE REGENERATIVE POTENTIAL OF SCHWANN CELL PRECURSORS AND SCHWANN CELLS DIFFERENTIATED FROM HUMAN IPSCS

Rebecca Powell[P][1,2], Parmjit Jat[3], Rickie Patani[4], Kristjan Jessen[5], James B. Phillips[1,2]

[1] *Department of Pharmacology, UCL School of Pharmacy*

[2] *UCL Centre for Nerve Engineering*

[3] *MRC Prion Unit at UCL, Institute of Prion Diseases*

[4] *The Francis Crick Insitute*

[5] *Department of Cell & Developmental Biology, UCL*

Corresponding author's email: ucbtpow@ucl.ac.uk

Background

Peripheral nerve injuries can lead to paralysis and chronic pain1. Tissue-engineered solutions use cells and biomaterials to improve peripheral nerve regeneration (PNR) without the drawbacks of the 'gold-standard' autograft 1–3. Aligned cellular collagen hydrogels using a range of cell types 4–7 support PNR. This may be improved by using Schwann cells (SCs) as they are key in the regeneration process8,9. Human iPSCs (hiPSCs) have been differentiated to SCs10 as human SCs are difficult to obtain and culture11,12. The aim of this project is to characterise and compare hiPSC-derived Schwann cells (SCs) with hiPSC-derived Schwann cell precursors (SCPs) in their ability to support peripheral nerve regeneration.

Methods

hiPSCs were expanded and differentiated into SCPs and SCs. RT-qPCR and immunocytochemistry (ICC) were used to characterise differentiated cells, with hiPSCs used as the control line. Viability and contraction assays of the cells in hydrogels were carried out.

Results

Oct4 gene expression significantly decreased between hiPSCs and SCPs, alongside Sox-10 significantly increasing. hiPSCs at high seeding density differentiated into SCPs expressing low levels of S100B. SCPs plated at low density differentiated further to SCs expressing high levels of S100B. These differentiated cells were positive for expression of SC markers Sox10 and P75. The differentiated cells had 80% viability in collagen hydrogels but limited ability to contract hydrogels.

Conclusions

Over 75% of differentiated hiPSCs expressed SC markers (mRNA and protein) and survived in collagen hydrogels. Neurite outgrowth assays and neurotrophic factor release will be compared to determine their ability to support peripheral nerve regeneration.

Keywords

Schwann cells; Nerve regeneration; Hydrogels

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Abstract **#879**

EFFECTS OF MACROPHAGE POPULATIONS ON VOCAL FOLD FIBROBLASTS REGENERATIVE BEHAVIOR

Sepideh Mohammadi$_P$[1], Fatemeh Taheri[1], Luc Mongeau[1]

[1] *Department of Mechanical Engineering, McGill University, Montreal, QC, Canada*

Corresponding author's email: sepideh.mohammadi@mail.mcgill.ca

Macrophages contribute to the elimination of pathogens, and also serve other roles in wound healing, angiogenesis, and organ regeneration [1]. According to recent studies, macrophages are responsible for modulating fibrosis and scarring during wound healing [2-4]. The present study aims to compare the effects of different macrophages activation states on the regenerative behavior of vocal fold fibroblasts. To this end, THP-1 monocytes were differentiated into inactivated macrophages (M0) through exposure to 200 nm phorbol 12-myristate 13-acetate. Macrophages were polarized by treatment with 20 ng/ml interferon-gamma and 100 ng/ml lipopolysaccharide for inflammatory macrophages (M1), 20 ng/ml Interleukin-4 and 20 ng/ml Interleukin-13 or 20 ng/ml Interleukin-10 for two subtypes of anti-inflammatory macrophages (M2a and M2c). A 3D co-culture system using two well silicon inserts allowed the encapsulation of fibroblast and macrophages separately in gelatin-based hydrogels. After 21 days, most of the fibroblasts co-cultured with M1 fibroblasts adopted a rounded morphology and produced a very low level of collagen type I (col-I) and collagen type III (col-III). The M2a group possessed aligned fibroblasts with significantly higher α-smooth muscle actin and col-I production compared to the other groups. The observations confirmed the contribution of M2a to wound healing via fibrosis. The fibroblasts affected by M2c showed an elongated spindle-like morphology similar to the M0 group. They also showed increased production of col-III, which is characteristic of scar-free wound healing. The results illustrate the potential benefits of immunomodulation for promoting tissue regeneration.

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Abstract **#880**

BUILDING HIERARCHY THROUGH VOXELATED MICROSYSTEMS IN 3D TO FORMULATE ADVANCED TISSUE CONSTRUCTS

Castro Johnbosco$_P$[1], Tom Kamperman[1], Jeroen Leijten[1]

[1] *Faculty of Science and Technology, Technical Medical Centre, Department of Developmental Bioengineering, University of Twente, Drienerlolaan 5, 7522 NB Enschede, Netherlands.*

Corresponding author's email: c.johnbosco@utwente.nl

Complex tissues are comprised of spatially arranged building blocks, which facilitate their collective behavior, enabling the existence of emergent properties that characterize the function of native tissues. In recent years, novel technological solutions have been sought to incorporate spatial complexity into engineered tissues. However, straightforward, scalable, and translatable solutions have remained wanted, to incorporate modularity in complex engineered living matter, especially those that are of clinically relevant size. Here, we propose engineered regulated single cell microgels as micro building blocks that can act as individually tuned voxels within a three-dimensional bulk system. Specifically, we exploited droplet microfluidics to encapsulate individual cells in biotinylated dextran-based microgels that could be further functionalized with moieties of interest via a cytocompatible and orthogonal crosslinking route. In bulk, these functionalizable single cell microgels formed a granular medium that could be processed via standard additive manufacturing approaches, thereby acting as tunable, printable, and living voxels. This approach effectively uncoupled the micro- and macro environment, which allowed the design space in which emergent properties can be created. Moreover, by selecting a biomaterial that allows on-demand biochemical and mechanical tunings, the substrate stiffness and composition of the microencapsulated cells could be controlled temporally. We demonstrated that this engineering strategy was compatible with additive manufacturing technologies such as bioprinting, which jointly offers novel and unique opportunities to engineer hierarchical structures that exhibit the collective behavior that drives the existence of emergent properties.

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Abstract **#882**

BIOMANUFACTURING OF PERFUSABLE ENGINEERED CARDIAC TISSUES

Sebastien Uzel$_P$[1,2], Mark Skylar-Scott[1,2,3], Aric Lu[1,2], Jennifer Lewis[1,2]

[1] *Harvard University*

[2] *Wyss Institute*

[3] *Stanford University*

Corresponding author's email: suzel@g.harvard.edu

Biomanufacturing functional human tissues and organs derived from patient-specific cells is a grand challenge. Specifically, the cellular density and organ-specific microarchitecture within these engineered constructs must be replicated to ensure functional outputs akin to their native counterparts. An embedded network of perfusable vessels is also needed to efficiently supply oxygen and nutrients needed to sustain bulk tissues. Building upon recent advances in patterning vascular networks [1–4] and generating multicellular aggregates [5–7], we recently reported a new biomanufacturing method, known as sacrificial writing into functional tissue (SWIFT) [8], which enables the rapid fabrication of densely cellular (>100 M cells/mL), vascularized cardiac tissues. To create the desired multiscale vasculature, we complemented this method by promoting the bottom-up assembly of microvascular features. While this talk will focus on engineered cardiac tissue, our integrated biomanufacturing platform can be broadly applied to creating functional organ-specific tissues at therapeutic scales.

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Abstract **#883**

COORDINATED BIO-ENGINEERED ALVEOLAR BONE/DENTAL IMPLANTS FOR REPAIR OF RABBIT MANDIBLE CRITICAL SIZED DEFECTS

Weibo Zhang$_P$[1], Zihan Li[1], Shruti Saxena[2], Amir Fakhrzadeh[2], Simon Young[3], Joachim Kohn[2], Pamela Yelick[1]

[1] *Department of Orthodontics, Tufts University School of Dental Medicine, Boston MA USA*

[2] *New Jersey Center for Biomaterials, Rutgers University, Piscataway NJ USA*

[3] *Department of Oral & Maxillofacial Surgery, The University of Texas Health Science Center at Houston School of Dentistry, Houston TX USA*

Corresponding author's email: weibo.zhang@tufts.edu

Craniomaxillofacial (CMF) defects remain a significant health concern. Current restoration of large CMF bone and tooth defects requires a two-staged surgical approach – bone repair followed by dental implant placement. The long-term goal of our research is to develop effective therapies for coordinated regeneration and functional repair of CMF defects that reduce surgical costs and patient recovery times. To achieve our goals, we tested a well-characterized tyrosine-derived polycarbonate scaffold, E1001(1K)-bTCP, for CMF repair in a critical sized rabbit mandible defect model. Our published results showed abundant jawbone formation using E1001(1K)-bTCP scaffolds seeded with human dental pulp cells (hDPCs), the ideal cell type for craniofacial bone and tooth regeneration. Here we further improved this approach using a modified E1001(1K)/dicalcium phosphate dihydrate (DCPD) scaffold supporting a dental implant. First, DCPD scaffolds with or without seeded hDPCs and HUVECs were cultured in osteogenic media for one week. A titanium (Ti) implant was screwed into each 10mm diameter x 6mm DCPD scaffold immediately before implantation. For each of 2 time points (1 and 3 months), 3 hDPC/HUVEC seeded and 2 acellular constructs were placed in full width, 10mm diameter mandibular defects, one per rabbit. MicroCT analysis was performed immediately after harvest, followed by histological and IHC analyses. Although ongoing, our preliminary analyses showed significant new bone formation in cell-seeded constructs, indicating the potential utility of hDPC/HUVEC seeded E1001(1K)-DCPD scaffolds for coordinated CMF bone and tooth regeneration.
These studies were supported by AFIRM2CF-04 W81-XWH-14-2-0004 (PCY) and NIH/NIDCR/NIBIB R01DE026731 (PCY).

Abstract **#884**

DEVELOPMENT OF 3D PRINTED SCAFFOLD FOR OSTEOCHONDRAL REGENERATION

Fiona Verisqa$_P$[1], Jae-ryung Cha[2], Linh Nguyen[1,3], Hae-Won Kim[4,5,3], Jonathan Knowles[1,3,6]

[1] *Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, United Kingdom*

[2] *Department of Chemistry, Dankook University, Cheonan, Republic of Korea*

[3] *UCL Eastman-Korea Dental Medicine Innovation Centre, Dankook University, Cheonan, Republic of Korea*

[4] *Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, Republic of Korea*

[5] *Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan, Republic of Korea*

[6] *The Discoveries Centre for Regenerative and Precision Medicine, University College London, United Kingdom*

Corresponding author's email: fiona.verisqa.19@ucl.ac.uk

Introduction: Osteochondral defects cause pain and disability in the human body. It is important to create a scaffold that mimics the cartilage and the subchondral bone as an alternative to reduce the risk of conventional treatment such as device failure, donor site morbidity, and host rejection to allografts. 3D printing is a method to fabricate a bilayer scaffold with an intricate structure that corresponds to the properties of each osteochondral layer. This study aims to develop a 3D printed bilayer scaffold to meet the requirement for osteochondral regeneration by using CSMA-2 for the bone layer and hydrogels as the candidate for the cartilage layer.

Methods: CSMA-2 scaffolds with gyroid design were printed by using Digital Light Processing (DLP) 3D printing method. MC3T3-E1 cells were seeded on the scaffold to evaluate its biocompatibility. In vitro metabolic activity, live cell visualisation, cell attachment, and spreading were analysed using Alamar blue assay, LIVE/DEAD assay, and SEM.

Results: The results showed that the gyroid structure with 400 µm pore size diameter was successfully printed by using CSMA-2 with the DLP 3D printing method. MC3T3-E1 cells remained viable after 14 days of incubation. The cells were found to attach and spread on the 3D printed scaffolds. The distribution of the cells also followed the gyroid architecture of the scaffold.

Discussion and Conclusions: CSMA-2 can be developed as bone layer material for the bilayer scaffold. This work will allow further development of the 3D printed biocompatible and functional scaffold with mechanical integrity, particularly under load-bearing conditions.

Abstract **#886**

IN VIVO CONDITIONS IN A CORNEAL BIOREACTOR IMPROVES THE FUNCTIONALITY OF ENGINEERED CORNEAL ENDOTHELIUM.

Princia Anney[P][1,2,3], Mathieu Thériault[1,2,3], Stéphanie Proulx[1,2,3]

[1] *Centre de recherche du CHU de Québec-Université Laval, axe médecine régénératrice*

[2] *Centre LOEX de l'Université Laval*

[3] *Département d'ophtalmologie, Faculté de médecine, Université Laval*

Corresponding author's email: PRANN@ulaval.ca

Context: The functionality of engineered corneal endothelium (ECE) is linked to the ability of the corneal endothelial cells (CECs) to form intercellular junctions. The purpose of this study is to assess the influence of intraocular pressure on the formation of intercellular junctions of CECs.Materials and methods: Cultured CECs were seeded on devitalized corneas and culture for 7 days to form ECE (n = 6 pairs). One of the ECEs was placed 7 days in a corneal bioreactor in the presence of a pressure of 16 mmHg, while the mate cornea was cultured without IOP. Morphology was assessed using cell circularity. The RNA was harvested, and gene expression of proteins associated with intercellular junctions was quantified by PCR. Results: IOP increased gene expression by 1.72 ± 0.31, 1.58 ± 0.41, 6.18 ± 1.03, 1.80 ± 0.71, 1.77 ± 0.55, 2.42 ± 0.71 for intercellular junctions related proteins gene GJA1, CDH2, TJP1, ITGAV, ITGB5 and CTNND1. CECs population circularity (0.66 ± 0.15, 0.77 ± 0.1, 0.62 ± 0.17, 0.82 ± 0.09, 0.74 ± 0.13 and 0.77 ± 0.12) correlated with the increase of mRNA transcription fold-change of TJP1and CTNND1, with r = 0,8305 for TJP1 and r = 0,5776 for CTNND1.Conclusion: These results indicate that the intraocular pressure increases the transcription of intercellular junctions-associated proteins. A better understanding of the genesis of CECs intercellular junctions can help to improve ECE functionality thus better tissue engineered corneas.

Abstract **#887**

TOWARDS A COMPLIANT, URINE RESISTANT AND REGENERATIVE ECM-DERIVED BIO-MATERIAL FOR NEO-BLADDER RECONSTRUCTION

Shivang Sharma[P][1], Sarah Rajani[2], Justin Sharma[2], Anirudha Singh[3]

[1] *Department of Chemical and Biomolecular Engineering, Johns Hopkins University*

[2] *Department of Biomedical Engineering, Johns Hopkins University*

[3] *Department of Urology, Johns Hopkins University*

Corresponding author's email: ssharm55@jhu.edu

ECM-derived biomaterials are biologically advantageous for wound healing, tissue remodeling, and regeneration, due to the presence of many inherent biologic molecules. Despite these benefits, they lack sufficient compliance (inverse of stiffness, or the ability to stretch under minimal applied force) required to reconstruct a mechanically functional compliant-tissue. Moreover, due to the lack of a urine-resistive mechanism in these scaffolds, the surrounding tissues are prone to urine toxicity, especially during the initial days of tissue remodeling. Here, we present a transient crosslinking-based biomaterial strategy that leads to the development of bladder-like compliance without the covalent crosslinking, simultaneously reducing the urine leakage into the surrounding peritoneal cavity. The resulting biomaterials displayed superior stability towards enzymatic degradation and mimicked native tissue compliance more closely. Additionally, the developed biomaterials exhibit a pro-regenerative in-vitro and in-vivo immune signature without any significant fibrotic response. This is significant as chemical modification, such as covalent crosslinking, commonly used to alter mechanical properties of ECM-based scaffolds, can cause biomaterial immunogenicity. Bladder augmentation in rats indicates that the regenerated tissue support regeneration of all the bladder wall components with a significant reduction in susceptibility towards the urine-stone formation. Here we will also present our recent data on the mechanical functionality of the regenerated bladder tissue in terms of contractility and its relationship to the single-cell transcriptomics of the bladder tissue regeneration process.

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Abstract **#888**

THE USE OF PROPOLIS FOR WOUND HEALING: A SCIENTOMETRIC ANALYSIS

Julia Carnelós Machado Velho$_P$[1], Thaís Amaral França[1], Maria Malagutti-Ferreira[1], Beatriz Kimberly Nath Kramer[1], Flavia Freddi Rodriguez[1], Ademilson Espencer Egea Soares[2], Murilo Racy Soares[3], João Tadeu Ribeiro-Paes[1]

[1] Department of Biotechnology, São Paulo State University - UNESP, Assis, São Paulo, Brazil

[2] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

[3] Department of Obstetrics and Gynecology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Corresponding author's email: juliamvelho@gmail.com

Regenerative medicine (RM) represents a new area of knowledge in biomedical sciences. The interaction of RM and tissue engineering (TE), currently referred by the acronym TERM, has allowed to optimize the development of biomaterials for insertion of cells or different types of molecules. Propolis is one of the most well-known antibacterial and anti-inflammatory agents used by humans since ancient times. Recently, studies have evaluated the effectiveness of propolis associated with biomaterials for wound healing. Therefore, the aim of this study was to conduct a scientometric analysis of the published studies about the use of propolis for wound healing. The data was obtained from the sites SCOPUS and Web of Science by searching the terms “Propolis and wound healing” in title, abstracts and keywords. We found 275 documents and, after filtering, 113 articles were evaluated. The results show that researches involving the use of propolis for wound healing has grown in recent years and about 70% of the studies have been developed in the last decade. Brazil is the main country in research about this subject and medicine, pharmacology and biochemistry were the most related subject areas. Moreover, we observed a recent trend to the association of propolis with biomaterials, mainly films and hydrogels, resulting in a better efficiency in wound healing. In conclusion, the analysis evidenced that the use of propolis is a promising therapeutic alternative for wound healing, since the vast majority of studies have shown that treatment with propolis is significantly more effective than control.

Keywords

Biomaterials; Wound healing; Regenerative medicine

Abstract **#889**

CHARACTERIZATION OF P16INK4A PROMOTER DRIVEN EXTRACELLULAR VESICLES PURIFIED BY STEVAC METHOD

Madhusudhan Reddy Bobbili[P][1,2], Markus Schosserer[2], Wolfgang Holnthoner[1], Heinz Redl[1], André Görgens[3], Samir EL Andaloussi[3], Johannes Grillari[1,2]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology*

[2] *University of Natural Resources and Life Sciences, Vienna*

[3] *Karolinska Institute*

Corresponding author's email: madhusudhan.bobbili@trauma.lbg.ac.at

Extracellular vesicles (EVs) are cell- derived lipid membrane nanoparticles that serve as messengers of intercellular communication, transferring bioactive molecules such as DNA, RNA, proteins and lipids to recipient cells. EVs have a natural therapeutic potential with high flexibility and biosafety for employing natural and synthetic biomolecules as therapeutic delivery vehicles. To get insights into the tissue-specific cargo in vivo for complete exploitation of EVs as therapeutic, biomarker and diagnostic tools, EV purification methods are critical. The aim of the study was brought about to develop an efficient EV purification method both in vitro and in vivo and to further investigate EVs as therapeutic targets in cellular senescence.

Firstly, to isolate tissue- specific EVs we developed Snorkel-tag based Extracellular Vesicle Affinity Chromatography (StEVAC). We systematically evaluated the purification of EVs harboring snorkel-tag by employing different methodologies. Our findings suggest that EVs harboring snorkel-tag indeed can be purified at high purity without altering EV characteristics and uptake. Furthermore, we expressed CD81-snorkel-tag under p16ink4a promoter and were able to purify EVs derived from senescent fibroblasts in vitro.

Finally, we are developing an in vivo model with recombinant CD81-snorkel-tag under p16ink4a promoter. This will provide us detail insights into the senescent cell derived EV cargo, allowing us to develop potential biomarker and therapeutic tools.

Summarized, we have here developed novel tool for studying content and function of EVs in the context of aging and disease. This will now pave the way for studying the molecular mechanisms underlying these EV functions in vivo.

Abstract **#890**

A MICROFLUIDIC APPROACH FOR ANALYZING THE EFFECT OF COMBINATORIAL FLOW FORCES ON VASCULAR NETWORK FORMATION

Fabian Steinp[1,2,3], Prasanna Padmanaban[1,2,3], Jeroen Rouwkema[1,2,3]

[1] *University of Twente*

[2] *Department of Biomechanical Engineering*

[3] *Vascularization Lab*

Corresponding author's email: j.rouwkema@utwente.nl

In the human body several different flow conditions could be identified in the vascular tree, depending on the vessel type, vessel size and that to be supplied tissue. Current microfluidic systems often use ranges of those fluid flow forces which are not mimicking accurately those conditions or only include a small range of forces known in vivo. To understand which fluid flow forces could potentially benefit the formation of a vascular network in engineered tissues it is necessary to analyze with high accuracy the combinatoric effect of different wall shear stress and the interstitial fluid velocities. In the present study, authors have developed a microfluidic system to study the combinatoric effect of five different wall shear stress simultaneously with a uniform constant interstitial flow velocity through the used fibrin hydrogel to mimic the flow conditions in vivo. For studying the effect of mechanical signals and the angiogenic growth factor (VEGF165) on vascular network formation, human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) were encapsulated within the hydrogel. The results obtained from initial COMSOL modelling and experiments confirmed a high control about different combinations of wall shear stress and transmural flow. Furthermore, the formation of vascular networks could be observed promoted by the interstitial fluid and differences in their structures were analyzed by image processing.

Keywords

Microfluidc; Vascular network formation; Fluid flow

Abstract **#891**

DECELLULARIZED MAN-MADE HYALINE CARTILAGE GRAFT FOR CARTILAGE TISSUE ENGINEERING

Dongan Wang$_P$[1,2]

[1] *City University of Hong Kong*

[2] *Karolinska Institute Ming Wai Lau Centre for Reparative Medicine*

Corresponding author's email: dwang229@cityu.edu.hk

Introduction: The key challenge of lower-limb-joint osteochondral regeneration lies in restoration of the avascular articular cartilage. Articular cartilage repair has been a significant challenge due to the limited self-regenerative capability of cartilage tissue. A quality articular cartilage engraftment is validated by the graft's hyaline cartilaginous phenotype and genuine microstructural architecture. Current treatments are frequently reported to result in regeneration of mechanically inferior fibrocartilage.

Experimental methods: In this study, we have developed a continuous methodology to directly set up a scaffold-free macro-scaled three-dimensional living hyaline cartilage graft (LhCG) with the aid of a biomaterial-based interim scaffolding system. The practical performance of allogeneic decellularized LhCG (dLhCG) is evaluated in the knees of pig models with full-thickness chondral defects beyond critical sizes for 6 months.

Results and discussions: Sound regeneration of articular hyaline cartilage via allogeneic dLhCG engraftment in 6 months after implantation has been shown, including the recoveries in form and function with correct composition, structure, phenotype and mechanical property.

Conclusions: The successful regeneration of articular cartilage defects in large animal models suggests the readiness of allogeneic dLhCG for clinical trials and applications.

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Abstract **#893**

DISEASE MODIFYING TREATMENT OF SPINAL CORD INJURY WITH DIRECTLY REPROGRAMMED NEURAL PRECURSOR CELLS IN NON-HUMAN PRIMATES

Vladimir P. Baklaushev[P][1], Vladimir A Kalsin[1], Gaukhar M Yusubalieva[1]

[1] *Federal Clinical and Research Center, FMBA of Russia*

Corresponding author's email: serpoff@gmail.com

We have previously described the development of a model of evoked potentials guided complete SCI on NHP (Macaca mulatta) that we used in the current study with the goal of investigating the safety and efficacy of intraspinal transplantation of directly reprogrammed neural precursor cells (drNPCs). Seven NHPs with verified complete thoracic SCI were divided into two groups: drNPC group (n=4) was subjected to intraspinal transplantation of 5 million drNPCs rostral and caudal to the lesion site two weeks post injury, and lesion control (LC, n=3) was injected identically with the equivalent volume of vehicle. Follow-up for 12 weeks revealed that animals in the drNPC group demonstrated a significant recovery of the paralyzed hindlimb as well as recovery of SSEP and MEP of injured pathways. MRI data confirmed the intraspinal transplantation of drNPCs did not adversely affect the morphology of the CNS or cerebrospinal fluid circulation. Subsequent immunohistochemical analysis showed that drNPCs maintained Sox2 expression characteristic of multipotency in the transplanted spinal cord for at least 12 weeks, migrating to areas of axon growth cones. Our data demonstrated that drNPCs transplantation was safe and contributed to improvement of spinal cord function after acute complete SCI, based on neurological status assessment and neurophysiological recovery within 12 weeks after transplantation. The functional improvement described was not associated with neuronal differentiation of the allogeneic drNPCs. Instead, directed drNPCs migration to the areas of active growth cone formation may provide exosome and paracrine trophic support, thereby further supporting the regeneration processes.

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Abstract **#894**

TIME-LAPSED MICROSTRUCTURAL IMAGING REVEALS INCREASED BONE MINERALIZATION IN POLYMER NANOCOMPOSITE SCAFFOLDS CULTURED UNDER CYCLIC LOADING IN DYNAMIC COMPRESSION BIOREACTORS

Gian Nutal Schädli[1,2], Jolanda R. Vetsch[1], Robert P. Baumann[2], Anke M. de Leeuw[1], Esther Wehrle[1], Marina Rubert[1], Ralph Müller[1]

[1] *Institute for Biomechanics, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland*

[2] *Particle Technology Laboratory, Department of Mechanical and Process Engineering, ETH Zurich, Zurich, Switzerland*

Corresponding author's email: giannutal.schaedli@hest.ethz.ch

Advancement in bone scaffolds that support mineralized tissue formation relies on biologically relevant animal studies. However, animal studies are cost-intensive and hardly scalable. Here, we present an in vitro bioreactor approach to monitor bone formation and mineralization in scaffolds under dynamic compression. Biomaterial processing of a resorbable polymer using a high-volume content of ultrasonically treated hydroxyapatite (HA) nanoparticles and a mixture with barium titanate (BT) was used to engineer mechanically competent bone scaffolds. The piezoelectric property of BT is attractive for tissue engineering due to its ability to deliver additional electrical stimulation under mechanical loading1. However, the high linear absorption property of BT requires advanced imaging protocols for comparison with other scaffold materials.The engineered scaffolds exhibited compressive moduli exceeding values of previous reports twofold, while maintaining their high porosity. After seeding with human bone marrow stromal cells, time-lapsed micro-CT imaging allowed longitudinal monitoring of scaffold mineral density (SMD), mineral volume and formation rates to assess the effect of cyclic loading on mineral maturation. After week 7, mineral volume in HA scaffolds under cyclic loading was 50% higher than under static conditions. Local bone formation rates corresponded to cell distributions. Longitudinal analysis of the SMD based on a single threshold showed a significantly higher maturation rate for scaffolds containing BT and HA under cyclic loading compared to static and to pure HA under dynamic or static conditions. This observation was corroborated by collagen and mineral stainings, suggesting scaffolds containing BT and HA as promising candidates for bone tissue engineering applications.

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Abstract **#895**

CONDUCTIVE ELASTOMERS FOR APPLICATIONS IN FLEXIBLE BIOELECTRONIC DEVICES

Estelle A. Cuttaz$_P$[1], Christopher A.R. Chapman[1], Josef A. Goding[1], Rylie A. Green[1]

[1] *Imperial College London*

Corresponding author's email: estelle.cuttaz18@imperial.ac.uk

Bioelectronic devices aim to stimulate and record neural activity to provide a therapeutic effect in neurological disorders [1],[2]. Conventional technologies utilise metals as electrode materials for interfacing with the nervous system. Metallic electrodes present serious limitations such as low charge injection limits [3] and high stiffness resulting in chronic inflammatory responses and scar tissue encapsulation. This ultimately hinders long-term device stability and performance. Soft, flexible fully polymeric bioelectronics have been proposed for addressing these limitations. This work explores the fabrication of soft and flexible polymer-based electrode arrays based on conductive elastomers (CEs). CEs are composites of poly(3,4-ethylenedioxythiophene) (PEDOT) and polyurethane elastomer. Flexible sheets of CEs with thicknesses ranging from 30 to 200 µm were produced by solvent casting dispersion of PEDOT particles within polyurethane, across varying PEDOT loadings. Electrochemical properties of the CE sheets were improved by increasing PEDOT content, resulting in lower impedance and increased charge storage capacities. Conductivities of CEs also improved with higher PEDOT amount, reaching up to 155.56 S.cm-1. In vitro cytocompatibility was assessed by cell growth inhibition assays. Bioelectronic device fabrication was done by laser micromachining the CE sheets and insulating with polydimethylsiloxane to produce flexible bipolar nerve cuff arrays. CE cuffs were able to stimulate ex vivo rat sciatic nerves and showed improved electrochemical performance over commercial metal-based cuffs. Overall, CEs resulted in a machinable and flexible conductive material. The fabrication of functional bioelectronic devices suggests that CEs are a promising alternative to metal-based bioelectronics with the potential for better chronic functionality.

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Abstract **#896**

HYPERACUTE SERUM AS A REGENERATIVE THERAPEUTIC IN IN VITRO MODELS OF OSTEOARTHRITIS

Isabel Olmos Calvo$_P$[1], Olga Kuten-Pella[1], Istvan Hornyak[1,2], Adel Hinsenkamp[1,2], Andrea de Luna[3], Karina Kramer[3], Stefan Nehrer[3], Zsombor Lacza[1,2,4]

[1] *OrthoSera GmbH, Krems an der Donau, Austria*

[2] *Dept. Translational Medicine, Semmelweis University, Budapest, Hungary*

[3] *Danube University Krems, Krems an der Donau, Austria*

[4] *Institute Sport and Health Sciences, University of Physical Education, Budapest, Hungary*

Corresponding author's email: is.olmos.ca@gmail.com

Autologous blood-derived products are known to be involved in musculoskeletal regeneration by promoting cell proliferation and modulating inflammation (1-3). By altering the cell-hostile microenvironment we are pursuing to stop or even reverse the degenerative process in the joints of osteoarthritic patients. Hyperacute serum is a blood-derived product that retains the regenerative potential of platelet-rich plasma (PRP) overcoming its variability disadvantages (4,5). Hyperacute serum is obtained by squeezing a fibrin clot; its composition has been compared to PRP showing that it better resembles cellular and molecular concentrations of in vitro and in vivo samples (6). To expand the potential therapeutic use, a new formulation method was established. The stable, sterile, antibody-deprived freeze-dried format enables allogenic therapy with a highly regenerative and standardized blood product. Our aim is to test its regenerative capacities in the context of osteoarthritis alone and in combination with hyaluronic acid (HA). Two-dimensional cultures of primary chondrocytes were used for XTT viability analysis showing that hyperacute serum or its combination with HA supports cell growth comparable to the gold standard fetal calf serum. Moreover, gene expression of genes involved in extracellular matrix maintenance (aggrecan, collagen type II, metalloproteinases 3 and 13), together with inflammatory and chondrogenic markers are quantified. Confocal microscopy of 3D chondrogenic cultures are used together with histology to better understand the regenerative potential of hyperacute serum. We conclude the combination product retains the regenerative potential of both components, ie. the blood derivative and hyaluronan, and serves a potential new therapeutic modality for degenerative joint disesases.

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Abstract **#897**

PIEZOELECTRIC 3D PLATFORM BASED ON HYDROGEL-POLY(VINYLIDENE FLUORIDE) MICROSPHERES FOR MESENCHYMAL STEM CELL OSTEOGENIC DIFFERENTIATION

Gloria Gallego-Ferrer[1,2], Maria Guillot-Ferriols$_P$[1,2], María Inmaculada García-Briega[1], Laia Tolosa[3], Senentxu Lanceros-Méndez[4,5,6], José Luis Gómez-Ribelles[1,2]

[1] *Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Valencia, Spain*

[2] *Biomedical Research Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Valencia, Spain*

[3] *Experimental Hepatology Unit, Health Research Institute La Fe (IIS La Fe), Valencia, Spain*

[4] *Centre/Department of Physics, Universidade Do Minho, Braga, Portugal*

[5] *BCMaterials, UPV/EHU Science Park, Leioa, Spain*

[6] *IKERBASQUE, Basque Foundation for Science, Bilbao, Spain*

Corresponding author's email: mariaguillot5@gmail.com

Bone's inherent piezoelectricity is a key factor in guiding mesenchymal stem cells (MSCs) differentiation towards the osteogenic lineage. Piezoelectric polymers, such as poly(vinylidene fluoride) (PVDF), combined with magnetostrictive nanoparticles (MNPs) provide a suitable approach for MSCs electro-mechanical stimulation. When applying an external magnetic field, MNPs deform, deforming the polymer and varying the surface charge due to the piezoelectric effect. Our approach is based on a 3D cell culture platform consisting of a gelatin hydrogel with embedded MSC and PVDF microspheres, with and without cobalt ferrite oxide (CFO) nanoparticles.Microspheres were produced by electrospray technique, favouring the presence of around 85 % of beta-phase, the most electroactive one, and a crystallinity degree around 60%, in both PVDF and PVDF-CFO microspheres. PVDF-CFO microspheres presented a 9% (w/w) content of MNPs.Absence of cytotoxicity of the 3D construct was studied by means of a live-dead assay after 24 hours. No significant differences in viability were observed, using a gelatin hydrogel as control. MSCs proliferation, in static and dynamic conditions, was studied by means of MTS assay up to 7 days. MSCs were able to proliferate in the 3D constructs in both situations and the presence of the magnetic field enhanced proliferation in the environments containing microspheres.These findings lead the way for future approaches in bone tissue engineering using a 3D piezoelectric environment to induce the osteogenic differentiation of MSCs.Acknowledgements: Spanish State Research Agency (AEI) (including Feder funds) PID2019-106099RB-C41 and -C43 / AEI / 10.13039/501100011033 projects and BES-2017-080398 FPI grant are acknowledged.

Keywords

poly(vinylidene fluoride); magnetostrictive nanoparticles; mesenchymal stem cells stimulation

Abstract **#898**

DENDRIPLEXES AS TOOLS FOR GENE DELIVERY

Carlos Peniche Silva[P][1], Nadia Roumans[1], Martijn van Griensven[1], Elizabeth Rosado Balmayor[2]
[1] CBITE, MERLN Institute for Technology - Inspired Regenerative Medicine, Maastricht University, The Netherlands
[2] IBE, MERLN Institute for Technology - Inspired Regenerative Medicine, Maastricht University, The Netherlands
Corresponding author's email: c.penichesilva@maastrichtuniversity.nl

Viruses are recognized as the most efficient transfection vectors available for gene therapy. However, they are immunogenic and cytotoxic. As alternatives, several types of non-viral vectors have been developed. Among them, dendrimers are an attractive choice that promises high transfection efficiency, low toxicity, and fast body elimination. The aim of this work was to investigate different generations polyamidoamine (PAMAM) dendrimers for gene delivery to human cells. We prepared different dendriplexes formulations using generations from G4 to G7 of PAMAM dendrimers and eGFP and metridia luciferase (MetLuc) DNA plasmids. Variables such as charge ratio (N/P), complexation time, the solvent used for complexation, and incubation time were optimized. HEK 293 and NIH 3T3 cells were successfully transfected with obtained dendriplexes and the transfection efficiencies and cytotoxicity were assessed. The maximum level of expression was achieved at 48 hours post transfection for all tested dendriplexes. PAMAM G4 and G5 and high N/P ratios (1:25 and 1:50) yielded the highest transfection efficiency. LDH assay revealed good biocompatibility of all tested dendriplexes, with values of cell viability higher than 94% at 72 hours post-transfection.
Our results illustrate the potential of G4 and G5 PAMAM dendrimers as non-viral vectors for gene transfer into human cells.

Abstract **#899**

ANTI-INFLAMMATORY, PRO-ANGIOGENIC AND OSTEOGENIC PROPERTIES OF CS SCAFFOLD FOR BONE FRACTURE TREATMENT

Alessandra Soriente[P][1], Soledàd Perèz Amodio[2,3], Ines Fasolino[1], Maria Grazia Raucci[1], Christian Demitri[4], Elisabeth Engel[2,3], Luigi Ambrosio[1]

[1] Institute of Polymers, Composites and Biomaterials - National Research Council (IPCB-CNR), Viale J.F. Kennedy 54 - Mostra d'Oltremare pad. 20, 80125, Naples, Italy

[2] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10-12, 08028, Barcelona, Spain

[3] Materials Science and Metallurgical Engineering, Polytechnic University of Catalonia (UPC), Eduard Maristany 16, 08019, Barcelona, Spain

[4] Department of Engineering for Innovation, University of Salento, Via Monteroni, 73100, Lecce, Italy

Corresponding author's email: alessandra.soriente@cnr.it

Several studies are aimed at developing systems based on natural and biocompatible polymers for bone tissue engineering. Here, we emphasized how the bio-activation of chitosan (CS)-based scaffolds by organic and inorganic signals is able to promote osteogenesis, angiogenesis and to modulate the inflammation response by using in vitro models to mimic bone fracture microenvironment. CS scaffolds by using two different approaches based on inorganic and organic compounds, were bio-activated respectively1. The expression of inflammatory mediators was investigated (TGF-β and IL-6). Additionally, to assess the effect of CS scaffold on angiogenesis, CD31 expression, cell adhesion, proliferation, migration and tube formation by HUVECs were detected. The results highlighted that inorganic and organic signals promote cell proliferation and differentiation without significant differences between the material groups. In particular, scaffolds bio-activated by using inorganic signals (hydroxyapatite nanoparticles) inhibit pro-inflammatory mediator's production (IL-1β and IL-6), induce anti-inflammatory cytokine generation (IL-10) and reduce nitric monoxide metabolites (nitrites). Conversely, scaffolds bio-activated by using organic signals (BMP-2 mimicking peptide) were able to decrease pro-inflammatory markers without any effect on anti-inflammatory cytokines levels and on nitrites. Furthermore, scaffolds promote angiogenesis by increasing cell proliferation, migration and tube formation with best results obtained for BMP based-scaffolds. Our results support the concept that CS biomaterials may be novel multi-target devices to treat bone related inflammation stimulating neo-vascularization of tissue-engineered constructs.

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Keywords

Chitosan/PEGDA based scaffolds; Angiogenesis; Osteogenesis

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Abstract **#900**

EFFECT OF NANOSTRUCTURED 2D BLACK PHOSPHORUS ON HUMAN PROSTATE CELL BEHAVIOR

Ines Fasolino$_P$[1], Alessandra Soriente[1], Maria Caporali[2], Manuel Serrano-Ruiz[2], Maurizio Peruzzini[2], Luigi Ambrosio[1], Maria Grazia Raucci[1]

[1] Institute of Polymers, Composites and Biomaterials – National Research Council (IPCB-CNR),Mostra d'Oltremare pad.20 - Viale J.F. Kennedy 54, 80125 Naples, Italy

[2] Institute of Chemistry of Organometallic Compounds – National Research Council (ICCOM-CNR),via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy

Corresponding author's email: ines.fasolino@cnr.it

INTRODUCTION: Brachytherapy is the most advanced radiotherapy strategy for the local treatment of prostate cancer and it consists in the placing of radioactive sources closed to the tumour side thus killing cancer cells. However, brachytherapy causes the same adverse effects of external-beam radiotherapy [1]. Therefore, alternative treatment approaches are claimed for enhancing radiotherapy effectiveness and reducing side effects. Nanostructured exfoliated black phosphorus (2D BP) represents a novel strategy for local cancer therapy because of its ability to induce singlet oxygen production and act as photosensitizer [2]. Hence, we evaluated 2D BP in vitro effect on healthy and cancer prostate cell behavior.
METHODS: 2D BP was obtained through liquid exfoliation. 2D BP effect on healthy and cancer prostate cell behaviors was analyzed by investigating cell viability, oxidative stress and inflammatory marker expression.
RESULTS: 2D BP inhibited prostate cancer cell proliferation, meanwhile promoted healthy prostate cell survival in vitro by modulating oxidative stress and immune response with and without near-infrared light (NIR)-irradiation.
CONCLUSIONS: Nanostructured 2D BP is able to inhibit in vitro prostate cancer cell survival and preserve healthy prostate cell vitality by controlling oxidative stress and immune response.

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This study has been supported by Materias srl and Progetto Premiale di Area Science Park "OPEN LAB - A System of Open Research Facilities". The authors also thank Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.

Keywords
Nanostructured few-layer black phosphorus ; Prostate anticancer treatment; Oxidative stress

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Abstract **#901**

BIOLOGICAL PROPERTIES OF BIOCOMPOSITES BASED ON IONIC LIQUIDS FOR BIOMEDICAL APPLICATIONS

Alessandra Soriente[P][1], Ines Fasolino[1], Giovanna Giuliana Buonocore[2], Maria Grazia Raucci[1], Luigi Ambrosio[1]

[1] Institute of Polymers, Composites and Biomaterials - National Research Council (IPCB-CNR), Viale J.F. Kennedy 54 - Mostra d'Oltremare pad. 20, 80125, Naples, Italy

[2] Institute of Polymers, Composites and Biomaterials - National Research Council (IPCB-CNR), UOS, P.le Enrico Fermi, 1 80055 Portici (NA).

Corresponding author's email: alessandra.soriente@cnr.it

Microorganisms can often adhere to biomaterials forming biofilms on foreign bodies. The incorporation of IL in biomaterials (i.e. synthetic or natural polymers1) allows to obtain innovative biocompatible materials able to inhibit biofilm formation. In the present work, the effect of IL incorporated in polymer materials on cell response (cell adhesion, proliferation and inflammation reaction) was investigated.

Biocomposites based on polymers and IL [CnMIm]Cl were prepared at different compositions. The polymers (i.e. chitosan, PLA, HDPE) were dissolved in appropriate solvent and the IL [CnMIm]Cl solution with different chain-lengths at different concentrations (0.125, 0.250, 0.500 and 2.00 wt%) was added. The films obtained were characterized to evaluate the biological properties by using murine fibroblast cell line (L929) and human Mesenchymal Stem Cells (hMSC). The cell–material interaction in terms of cell biocompatibility and inflammation reaction was analyzed.

Results on cytotoxicity demonstrated that composites could be used as polymers for biomedical applications. The effect of the polymer-based biomaterials with different contents of ILs on hMSC behavior has confirmed a good biocompatibility. The study suggested that ILs incorporated in polymers provides access to biomaterials promising for the development of medical devices.

ACKNOWLEDGEMENTS

The study was supported by OPEN LAB. The authors thank Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.

Keywords

Biofilm formation; Ionic liquids; Biocompatibility

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Abstract **#902**

OSTEOGENIC AND ANTI-INFLAMMATORY PROPERTIES OF INJECTABLE CALCIUM PHOSPHATE LOADED WITH THERAPEUTIC DRUGS

Ines Fasolino$_P$[1], Alessandra Soriente[1], Luigi Ambrosio[1], Maria Grazia Raucci[1]

[1] *Institute of Polymers, Composites and Biomaterials – National Research Council (IPCB-CNR), Mostra d'Oltremare pad.20 - Viale J.F. Kennedy 54, 80125 Naples, Italy*

Corresponding author's email: ines.fasolino@cnr.it

INTRODUCTION: Actually, osteoporosis standard therapy consists in the systemic administration of biphosphonates and anti-inflammatory drugs, which cause several side effects [1]. In this scenario, the development of injectable materials loaded with therapeutically active agents could represent an effective strategy for locally treating bone loss and inflammation related to musculoskeletal diseases [2]. Here, we developed three different compositions of injectable Calcium Phosphates (CaP) as new carrier materials of therapeutic compounds such as bisphosphonates (i.e. Alendronate), anti-inflammatory drugs (i.e. Diclofenac) and natural molecules (i.e. Harpagoside), for the local bone disease treatment.
METHODS: Biological quantitative analyses were performed for screening osteoinductive and anti-inflammatory properties of injectable drug-loaded systems. Meanwhile, cell morphological features were analyzed through SEM and confocal investigations.
RESULTS: The results exhibited that the three systems (Alendronate-loaded CaP, Diclofenac-loaded CaP and HR-loaded CaP) exerted osteoinductive effect during later phases of osteogenesis. Simultaneously, all compositions showed an anti-inflammatory activity on inflammation in vitro models.

CONCLUSIONS: In conclusion, the three systems displayed beneficial osteogenic and anti-inflammatory properties in vitro, thus representing a multi-target strategy for the local therapy of inflammation and bone loss determined by osteoporosis.

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This study was supported by JoinTherapeutics srl and Progetto Premiale di Area Science Park "OPEN LAB - A System of Open Research Facilities". The authors also thank Mariarosaria Bonetti for lab technical support & data elaboration and Dr. Roberta Marzella for support to project management.

Keywords
Injectable drug loaded biomaterials; Bone tissue regeneration; Sol–gel method and in vitro model

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Abstract **#903**

ANTIMICROBIAL PHOTODYNAMIC THERAPY AGAINST POLYMICROBIAL INFECTIONS TO ENHANCE WOUND HEALING

Lisa Karner[P][1], Magdalena Metzger[1], Susanne Drechsler[1], Ara Hacobian[1], Barbara Schaedl[1,2,3], Paul Slezak[1], Johannes Grillari[1], Peter Dungel[1]

[1] *Ludwig Boltzmann Institute for experimental and clinical Traumatology, Vienna*

[2] *University Clinic of Dentistry, Medical University of Vienna*

[3] *Austrian Cluster for Tissue Regeneration, Vienna*

Corresponding author's email: peter.dungel@trauma.lbg.ac.at

The rapid establishment of antibiotic resistance in bacteria demands new therapies for the treatment of wound infections. A promising option is the combination of light with a photoreactive substance termed antimicrobial photodynamic therapy (aPDT). In this study the effectiveness of aPDT was tested as a treatment option for polymicrobially infected wounds in a new developed infection model.

A new pre-clinical wound healing model was established in immunocompromised BALB/c mice based on fecal bacteria. PDT was applied twice within 24h using 100 µM methylene blue (generally regarded safe for topical application on human skin) and 24 J/cm2 pulsed red LED light (Repuls). The effects of aPDT were analyzed by selected quantitative methods for wound analysis like wound area, wound score, microbiological analysis and imaging techniques.

Polymicrobial infection significantly delayed wound healing leading to larger and slower closing wounds. A newly established wound score, assessing the severity of wounds and the establishment of infection based on clinical monitoring, confirmed the infection-induced delay in wound healing. Wound healing of infected wounds was drastically enhanced by aPDT treatment with methylene blue.

Wound infections are challenging for patients and nursing staff, but also for medical research, especially with the increasing incidence of multidrug-resistant bacteria. The new model presented in this study with appropriate analysis tools provides the means to investigate complex microbiological interactions and new therapy approaches. Our data suggest that aPDT has the potential to significantly improve wound healing also in complicated polymicrobially infected wound situations.

Abstract **#904**

PLURIPOTENT STEM CELLS FOR DRUG DEVELOPMENT AND THERAPY: BIOPROCESS DEVELOPMENT CHALLENGES

Paula M Alves$_P$[1,2]

[1] *IBET - Instituto de Biologia Experimental e Tecnologica*

[2] *ITQB NOVA - Inst Tecnologia Quimica e Biológica New Univ Lisbon*

Corresponding author's email: marques@ibet.pt

Human pluripotent Stem Cells (hPSC) have an enormous potential as a source for cell replacement therapies, tissue engineering, and in vitro toxicology applications. The lack of standardized and robust bioprocesses for their expansion and analytical tools for their characterization has hindered the application of PSC in clinical settings and as alternative cell sources for in vitro cell models used in pre-clinical research [1]. Bioengineering approaches exploiting the potential of 3D cell culture models and scalable perfusion bioreactor technology for the production of hPSC will be discussed together with the application of -omic tools for characterization of the cell-based products [2,3]. A focus will be given to integrative approaches that allow the identification of metabolic hallmarks to support bioprocess developmentResults concerning the establishment and refinement of culture systems for efficient stem cell differentiation and maturation into cardiac, neural and hepatic cells, as well as their applicability for long-term toxicity testing will be presented.

Keywords

Scalable and cGMP Manufacturing Stem Cells; Analitycal Tool for Characterization Cell BAsed Products; Bioprocess development & integration

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Abstract **#906**

BONE TISSUE ENGINEERING WITH RECOMBINANT HUMAN BMP-7 PROTEIN DECORATED ELECTROSPUN PCL FIBER GENERATED BY THE LBL TECHNIQUE

Luiz Henrique Catalani[P][1], Elayaraja Kolanthai[1], Ana Claudia Oliveira Carreira[2], Mari Cleide Sogayar[2,1]

[1] *Institute of Chemistry, University of São Paulo*

[2] *Medical School, University of São Paulo*

Corresponding author's email: catalani@usp.br

The combination of controlled growth factor delivery and physical support for cell growth onto biodegradable three-dimensional scaffolds (3D) established a notable change in the tissue engineering field. The objective of this work was to develop an electrospun 3D scaffold with the capability of controlled release of recombinant human bone morphogenetic protein-7 (rhBMP-7) for enhancement of bone tissue regeneration. Here, the rhBMP-7 protein was loaded to the surface of 3D PCL scaffold coated by the Layer-by-Layer (LbL) technique, with heparin and chitosan as the polyelectrolyte pair [1]. The physicochemical properties, such as surface morphology, functional group, thermal properties, and wettability of these protein reservoirs, were examined using different techniques, such as SEM, FTIR, TGA, contact angle measurement, and immunofluorescence. No changes in morphology and porosity of the fiber mat were observed upon LbL growth. The hydrophobic PCL surface was rendered hydrophilic upon the addition of polyelectrolytes, with protein coating over the surface. PCL coating with 50 ug/mL rhBMP-7, along with polyelectrolytes, was confirmed by immunofluorescence. The in vitro release profile of rhBMP-7 indicated an initial burst release, followed by a controlled release, measured by ELISA. Pre-osteoblastic C2C12 cells were cultured on scaffolds constructed with rhBMP7 reservoirs to assess cell attachment, proliferation, and differentiation under in vitro conditions. In vivo, rhBMP-7 delivered from electrospun scaffolds induced significant new bone formation. Therefore, this study presents a new approach to control both the loading and unloading of signaling proteins from biodegradable implants at infected tissue sites.

Keywords

BMP-7; electrospinning; Layer-by-Layer

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Abstract **#907**

3D CELL CULTURE IMAGING USING HYBRID LEARNING ASSISTED MINIATURE ELECTRICAL IMPEDANCE TOMOGRAPHY

Zhou Chen[1], Yunjie Yang[1], Pierre Bagnaninchi[2]

[1] *Agile Tomography Group, School of Engineering, University of Edinburgh, Edinburgh, UK*

[2] *MRC Regenerative Medicine Center, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, UK*

Corresponding author's email: Zhou.Chen@ed.ac.uk

In tissue engineering, miniature Electrical Impedance Tomography (mEIT) is an emerging tomographic modality that contributes to non-destructive and label-free imaging and monitoring of 3D cellular dynamics [1]. It visualises the cross-section conductivity distribution within the Region of Interest (ROI) by measuring differential voltages on the boundary. The main challenge of mEIT comes from the nonlinear and ill-posed image reconstruction problem, leading to the increased sensitivity to imperfect measurement signals [2]. Physical model-based image reconstruction methods [3-7] have been successfully applied to conventional setup, but are less satisfying for the mEIT setup in terms of image quality, conductivity retrieval and computational efficiency. Learning-based methods are a new frontier of EIT image reconstruction, which have been investigated on single-level conductivity variation between the homogeneous background and imaging objects [8,9]. However, in practice, continuous, multi-level conductivity variations have wider presence in 3D cell culture imaging. In this paper, we propose a deep learning and physical model based hybrid image reconstruction framework to enable high-quality cell culture imaging with mEIT. We validate our approach by imaging 3D cancer cell spheroids (MCF-7). Our method can be readily translated to spheroids, organoids culture and cell culture in biomaterials scaffolds. As the measured conductivity is a proxy for cell viability, mEIT has a great potential to enable non-invasive, real-time, long-term monitoring of 3D cell growth, opening new avenues in regenerative medicine and drug testing.

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Abstract **#908**

CUSTOM- FIT HARD TISSUE IMPLANTS FROM THE SYNTHESIS AND 3D PRINTING OF A NOVEL, LIGHT-CURABLE, DEGRADABLE POLYMER

Taleen Shakouri[P][1], Elena García- Gareta[2], Jonathan C Knowles[1]

[1] *Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, UK*

[2] *Regenerative Biomaterials Group, The RAFT Institute & The Griffin Institute, Northwick Park and Saint Mark's Hospitals, Harrow, UK*

Corresponding author's email: taleen.shakouri.18@ucl.ac.uk

For the correction of congenital defects, the removal of oral tumours and the reconstruction of the head and neck region, the surgeon is usually limited with devices available to restore both function and appearance for the patient. With the ever-increasing demand for low-priced efficient facial implants, there is an urgent need to advance new manufacturing approaches and implants with a higher osteointegration performance. Three-dimensional (3D) printing has the ability to enhance the production of on-demand fabrication of patient-specific devices as well as anatomically fitting implants with high complexity in a cost-effective manner. In recent years, the use of additive systems that employ vat photopolymerization such as stereolithography (SLA) and digital light projection (DLP) have been used widely in the field of biomedical science and engineering. However, additive manufacturing methods can be limited by the types of materials that can be used. In this study, we present an isosorbide-based formulation for a polymer yielding a range of elastic moduli between 1.7-3 GN/mm2 dependent on the photoinitiator system used as well as the amount of calcium phosphate filler added. The monomer was prepared and enhanced for 3D-printing using an SLA technique that delivered stable and optimized 3D- printed models. The resin discussed could potentially be used following major surgery in replacement of current materials and techniques used.

Keywords

Biomaterials; 3D Printing; Tissue Engineering

Abstract **#909**

VARIABILITY OF EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STROMAL CELL CLONAL SUBPOPULATIONS: IMPLICATIONS FOR CLINICAL USE

Savvas Ioannou[1], Andrew Stone[1], David Kuntin[1], Chris MacDonald[1], Paul G. Genever[1]
[1] *University of York, Department of Biology*
Corresponding author's email: si665@york.ac.uk

The use of extracellular vesicles (EVs) as acellular alternatives to cell-based therapies demands a defined EV population with a consistent bioactive cargo. Mesenchymal stem/stromal cells (MSCs) are an attractive source for therapeutic EVs, but they are highly heterogeneous which questions the quality and reproducibility of the EVs they produce. We have identified a biomarker (CD317) that discriminates human MSC subpopulations with regenerative properties (CD317-) from differentiation-incompetent/pro-inflammatory MSCs (CD317+). We investigated the morphological features, protein/miRNA content and functional activity of EVs isolated from CD317- and CD317+ clonal MSCs to help define appropriate therapeutic routes.
EVs isolated from CD317- and CD317+ MSCs by ultracentrifugation (100K fraction) had broadly similar peak average sizes (nanoparticle tracking analysis) and morphological characteristics (diameter, perimeter, area, roundness; TEM image analysis). However, using LC-MS/MS we identified a significantly enhanced EVome in CD317- compared to CD317+ MSCs (68 versus 2 upregulated proteins, $p<0.01$), and NanoString miRNA quantification identified 9 significantly upregulated ($q<0.05$) miRNAs in CD317- MSCs versus 2 in CD317+ MSCs. Bioinformatic analysis of proteomic and miRNA target data demonstrated functional links to cell migration/matrix interactions in the CD317- population, with a specific enrichment for PI3K-Akt signaling compared to CD317+ MSCs. We showed by fluorescent tracking that CFSE-labelled EVs from CD317- regenerative MSCs were taken up by primary MSCs and stimulated cell numbers (1.4-1.8 fold change compared to controls, n=3 biological replicates) determined by alamarBlue quantification.
These findings demonstrate the need to develop acellular regenerative therapies using defined EV populations and cell sources to optimise clinical outcome.

Keywords
Extracellular Vesicles; Mesenchymal Stromal Cells

Abstract **#910**

FUNCTIONAL CARDIAC ORGANOIDS MODELING THE INFLAMMATORY ENVIRONMENT OF MYOCARDIAL INFARCTION

Meenakshi Sukup[1,2,3], Manus Biggs[3], Michael Monaghan[1,2,3]

[1] *Trinity Centre for Biomedical Engineering, Trinity College Dublin, Ireland*

[2] *Department of Mechanical and Manufacturing and Biomedical Engineering, Trinity College Dublin, Ireland*

[3] *CURAM, National University of Ireland, Galway*

Corresponding author's email: sukum@tcd.ie

INTRODUCTION: Myocardial infarction (MI) is an ischemic and inflammatory event majorly orchestrated by macrophages from infiltrating monocytes (1). These macrophages play a critical role in deciding the fate of the heart post-MI (2). However, there is no cardiac disease model in existence that incorporates an appropriate inflammatory environment. Hence, the aim of this project is to develop a humanised model of MI, using induced pluripotent stem cell (iPSC) derived cardiomyocytes together with inflammatory cytokine stimulation, to model the disease environment. The first objective of this project is to obtain immune cell-conditioned media for stimulating cardiomyocytes, followed by developing a hydrogel platform to model MI.

METHODS: In order to achieve the first objective, iPSCs were expanded and cultured on different platforms such as ultra-low attachment plates (ULA), agarose microwell plate (AP) and Aggrewell plate (AW) to identify optimal conditions for differentiating them into immune cell lineages.

RESULTS: ULA, AP and AW plates successfully facilitated embryoid body (EB) formation. The sizes of EBs were more uniform in AP and AW as opposed to ULA.

DISCUSSION & CONCLUSIONS: Comprehensively, according to the preliminary data, ULA, AP and AW facilitate EB formation and differentiation into immune cell lineages. In order to determine the ideal hydrogel candidate for modelling MI, collagen-matrigel and collagen-fibrin hydrogels are being studied.

ACKNOWLEDGEMENTS: The authors acknowledge SFI for the funding (18/EPSRC - CDT/3583) and lifETIME CDT.

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Abstract **#911**

HUMAN ORGAN-ON-A-CHIP MODEL FOR PREDICTIVE DRUG SCREENING TO DETERMINE ANTI-TUMOR EFFICACY AND CARDIAC SAFETY

Alan Chramiec$_P$[1], Diogo Teles[1,2], Keith Yeager[1], Alessandro Marturano-Kruik[1,3], Joseph Pak[1], Timothy Chen[1], Luke Hao[1], Miranda Wang[1], Roberta Lock[1], Daniel Naveed Tavakol[1], Marcus Busub Lee[1], Kacey Ronaldson-Bouchard[1], Jinho Kim[4], Gordana Vunjak-Novakovic[1]

[1] *Columbia University*

[2] *University of Minho*

[3] *Politecnico de Milano*

[4] *Stevens Institute of Technology*

Corresponding author's email: ac3904@cumc.columbia.edu

Preclinical models are often unable to faithfully recapitulate human physiology. Organ-on-a-chip platforms that are being developed to overcome these shortcomings remain limited by their dependence on materials that are highly absorbent to drugs. We report the design and utilization of a novel, integrated, open setting, drug-binding free, tumor-heart tissue platform that allows for simultaneous assessment of the efficacy and cardiotoxicity of anti-cancer drugs. Previously, we established bioengineered human tissue models of Ewing sarcoma (ES) and cardiac muscle: the former recapitulated critical tumor microenvironmental phenotypes, while the latter emulated aspects of adult-like cardiac physiology and function. The two bioengineered tissues were studied in isolation and in the integrated platform following administration of linsitinib, a chemotherapeutic recently evaluated in clinical trials, according to the 3 week-long clinical regimen. Only the engineered tumor tissues, and not monolayers, recapitulated the clinically-relevant differences in drug responses between non-metastatic and metastatic ES tumors. More importantly, the responses of non-metastatic ES tissues and heart muscle to linsitinib were much closer to those observed in the clinical trial for tissues cultured in the integrated platform than those in isolation. Specifically, drug treatment of isolated tissues showed decreases in tumor viability and increases in both proarrhythmic events and beat frequency not seen in patients. Meanwhile, drug treatment via circulation in the platform showed poor tumor response and significantly milder cardiotoxicity, which matched the results of the clinical trial. Overall, the integration of the tissues in the platform improved predictive accuracy for both the direct and off-target effects of linsitinib.

Abstract **#912**

RENAL PROTECTIVE EFFECT OF BELUGA LENTIL PRETREATMENT FOR ISCHEMIA-REPERFUSION INJURY

EunHye Lee$_P$[1], So Young Chun[2], Bomi Kim[1], BoHyun Yoon[1], Byung Ik Jang[3], Dong Jin Park[4], Jun Nyung Lee[4], Man-Hoon Han[5], Bum Soo Kim[4], Phil Hyun Song[6], Dong Woo Lee[4], Eun Sang Yoo[4], Tae Gyun Kwon[4], Yun-Sok Ha[4]

[1] *Joint Institution of Regenerative Medicine, Kyungpook National University Hospital*

[2] *BioMedical Research Institute, Kyungpook National University Hospital*

[3] *Department of Internal Medicine, Yeungnam University College of Medicine*

[4] *Department of Urology, School of Medicine, Kyungpook National University*

[5] *Department of Pathology, School of Medicine, Kyungpook National University*

[6] *Department of Urology, Yeungnam University College of Medicine*

Corresponding author's email: eun90hye@gmail.com

Ischemia/reperfusion (I/R) injury, caused by acute kidney damage, causes histopathological alterations, tubule cell apoptosis, inflammation, oxidation, and loss of renal function. We evaluated the protective effects against IR injury of beluga lentil pretreatment. Mice were divided into four groups: normal, untreated, low- and high-dose beluga lentil treatment groups. Beluga lentil was orally administered for 2 weeks, followed by bilateral renal ischemia for 20 min and reperfusion for 30 min. Blood and kidneys were collected and analyzed to investigate renal function, histopathology, epithelial and endothelial cell damage, apoptosis, oxidative stress, and inflammatory responses. The pretreated groups maintained renal function, with significantly lower blood urea nitrogen (BUN) and creatinine levels, compared with the other groups. The histopathological analysis showed reduced proximal tubule injury and decreased injury-related molecule (KIM-1 and NGAL) secretion in the pretreated groups compared with the other groups. TUNEL-positive cells and the secretion of apoptosis-related molecules (Fas and caspase-3) was significantly reduced in the pretreated groups compared with the other groups. The pretreated groups showed positive microvessel-associated gene (CD31) expression and negative adhesion molecule (ICAM-1) expression. An antioxidant effect was observed in the pretreatment groups, with reduced malonaldehyde (MDA) expression and increased antioxidant enzyme (SOD, CAT, GSH, and GPx) secretion. In the pretreated groups, F4/80+ macrophages and CD4+ T cell infiltration were inhibited and pro-inflammatory cytokine (IL-1β, IL-6, and TNF-α) levels decreased; however, the levels of anti-inflammatory cytokines (TGF-β, IL-10, and IL-22 increased. Beluga lentil pretreatment demonstrated protective effects against IR induced renal damage, via anti-apoptotic, anti-inflammatory, and antioxidant activities.

Keywords

Kidney; Ischemia; Renal protection

Abstract **#913**

NANOPARTICLE-MEDIATED RELEASE OF TGF-B3 ENHANCES CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STROMAL CELLS

Yvonne Roger[P][1], Leonie Berten-Schunk[2], Alexander Becker[3], Johannes Pitts[2], Nils Meier[2], Heike Bunjes[4], Birgit Glasmacher[3], Henning Menzel[2], Andrea Hoffmann[1]

[1] *Hannover Medical School*

[2] *Technical University Braunschweig*

[3] *Leibniz University Hannover*

[4] *Technical University Brauschweig*

Corresponding author's email: Roger.Yvonne@mh-hannover.de

Tissue transitions like tendon bone junctions (enthesis) consist of a variety of features including gradients of properties with specific characteristics e.g. in structure as well as in composition and mechanical characteristics. At present there are limitations of implants with respect to such notably inhomogenous materials. Electrospun poly (ε-caprolactone) (PCL) fiber mats may be considered as a promising material for this kind of tissue engineering. They can be hydrophilized for cyto- and biocompatibility, e.g. by modification with a chitosan graft with PCL. This allows further modifications by nanoparticular drug-delivery systems. In order to achieve a controlled delivery of bioactive factors such as drugs or proteins to a specific region in vivo, nanoparticles are a promising option. Here we used chitosan/tripolyphosphate nanoparticles loaded with recombinant (E. coli) human TGF-β3. The transforming growth factor superfamily, including TGF-βs and BMPs, is involved in various cellular processes such as proliferation, differentiation and development. The release of TGF-β3 from the nanoparticle-coated fiber mats induced chondrogenesis in human bone marrow-derived mesenchymal stromal cells (MSCs). These findings make modified fiber mats in combination with the release of relevant factors a promising candidate for implants bridging complex tissue transitions.

Abstract **#914**

DEXAMETHASONE: A FRIEND OR A FOE IN OSTEOGENIC DIFFERENTIATION OF HUMAN BMSCS?

Elena Della Bella[P][1], Paras Ahmad[1], Antoine Buetti-Dinh[2], Ginevra Licandro[3], Valentina Basoli[1], Mauro Alini[1], Martin Stoddart[1]

[1] *AO Research Institute, 7270 Davos, Switzerland*

[2] *Laboratory of applied microbiology (LMA), Department of Environment, Constructions and Design (DACD), University of Applied Sciences of Southern Switzerland (SUPSI), 6500 Bellinzona, Switzerland*

[3] *Dalle Molle Institute for Artificial Intelligence (IDSIA), University of Italian Switzerland (USI), University of Applied Science and Art of Southern Switzerland (SUPSI), 6928 Manno, Switzerland*

Corresponding author's email: elena.dellabella@aofoundation.org

Dexamethasone is commonly used to induce osteogenic differentiation of bone marrow mesenchymal stromal cells (BMSCs). However, the rationale for its use is not clear considering that dexamethasone is also employed for chondrogenic and adipogenic differentiation, it is known to have a strong adipogenic effect, and its use both in vitro and in vivo is associated to reduced mineralization and maturation of osteoblasts. This work aimed then to clarify the role of dexamethasone during in vitro osteogenesis.

Human BMSCs were induced to osteogenic differentiation using a combination of concentrations of dexamethasone and time points of exposure. Biochemical characterization and gene expression analysis showed that the presence of dexamethasone on one side repressed SOX9 expression (hence improving osteogenic differentiation), but also increased PPARG expression with consequent development of pre-adipocyte-like cells within mineralizing cultures. RUNX2 expression was unaffected by dexamethasone, contrarily to previous reports.

Based on these results, we have introduced dexamethasone in a computational model describing the interaction between RUNX2, SOX9, and PPARG [1]. Thus, we used approximate Bayesian computation (ABC) simulations to estimate the parameters of the theoretical model that best fit the experimental measure of the activity of the three transcription factors. The simulations successfully reproduced the trends that were observed experimentally. However, the simulations also predicted a lower RUNX2 activity compared to experimental gene expression data, indicating that further signalling components are necessary for a better convergence of the model to the experimental observations.

Keywords

dexamethasone and osteogenic differentiation of BMSCs; approximate bayesian computation; transcription factors

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Abstract **#915**

LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1 PLAYS A KEY ROLE IN CHONDROCYTE DIFFERENTIATION AND PROLIFERATION

Wenjin Yan[P][1]

[1] *State Key Laboratory of Pharmaceutical Biotechnology, Department of Sports Medicine and Adult Reconstructive Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, 321 Zhongshan Road, Nanjing 210008, Jiangsu, PR China.*

Corresponding author's email: yanwenjin@njglyy.com

Low-density lipoprotein receptor-related protein 1 (LRP1) is expressed at a high level in articular chondrocytes, and the articular chondrocytes and synoviocytes in osteoarthritis have decreased amounts of LRP1 [1-3]. However, the underlying mechanisms and functional consequences for disease development remain poorly understood.

We identify extensive rare LRP1 variants in 126 severe developmental dysplasia of the hip (DDH) patients. By combining a WES high-throughput analysis with a comprehensive DNA screening, we targeting 16 rare LRP1 variants. By establishing a mouse line with Lrp1 gene knock-out mice via CRISPR-Cas9 System and blocking Lrp1 by using shRNA treatment in ATDC5, we discover an unexpected critical role for Lrp1 influencing chondrocyte differentiation and proliferation.

We evaluated the expression of chondrogenic differentiation markers by using real-time PCR and western-blot in ATDC5 on day 7, 14, 21. Sox9, Runx2 and Col2a1 was significantly decreased in Lrp1 knock down group. And after the day 28, proliferation of chondrocytes was also significant decreased. Lrp1 gene knock-out mice tends to grow with smaller acetabular volume and the “Y” cartilage closes earlier. The change of acetabular morphology will caused the decrease of acetabular volume which will lead to the increase of local stress on the articular surface and increase the instability of the hip joint.

Taken together, our results suggest that LRP1 plays a key role in chondrocyte differentiation and proliferation.

Keywords: LRP1, chondrocyte, developmental dysplasia

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Abstract **#916**

DEVELOPMENT OF AN IMMUNOPROTECTIVE MACROENCAPSULATION DEVICE FOR BETA CELL REPLACEMENT THERAPY IN TYPE 1 DIABETES

Denise de Bontp[1], Sami Mohammed[1], Omar Paulino da Silva Filho[1], Rick de Vries[1], Martijn van Griensven[1], Marlon Jetten[1], Vijayganapathy Vaithilingam[1], Marten Engelse[2], Eelco de Koning[2,3], Aart van Apeldoorn[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine - Cell Biology-Inspired Tissue Regeneration (cBITE)*

[2] *Leiden University Medical Center*

[3] *Hubrecht Institute*

Corresponding author's email: d.debont@maastrichtuniversity.nl

During Clinical Islet Transplantation (CIT) donor islets are transplanted via the portal vein and transfused into the hepatic vasculature. Although the liver is still considered the best-case scenario, the efficiency of this procedure is low because of allo- and autoimmune reactions, and risky due to the use of systemic immunosuppressive therapy. We report on the development of an immunoprotective macroencapsulation device for islets to omit the use of immunosuppressive drugs.

Thin and porous membranes were produced by a customized automated solvent-non solvent casting method from polyvinylidene fluoride (PVDF) in dimethyl-acetamide (DMA) and optionally supplemented with a water-soluble factor, polyvinylpyrrolidone (PVP). Encapsulation devices are made by fixating two membrane sheets. The membrane characteristics were determined by analyzing diffusion kinetics of different relevant molecules such as glucose. Immunoprotective properties were confirmed in both membranes with a macrophage migration assay. Human islets were cultured for 7 days inside the devices. The islet viability remained high over time in all conditions with viability over 90%. Islet function was assessed with a glucose-stimulated insulin secretion assay. Inside the devices, islets responded to changing glucose levels by releasing insulin, quantified by the stimulation index being larger than 2. PVDF/PVP devices had a higher stimulation index compared to PVDF devices.

We conclude that PVDF/PVP devices outperform PVDF devices with respect diffusion and the islet stimulation index. The immunoprotective PVDF/PVP device has the potential to improve the efficiency of current CIT.

Abstract **#918**

ENGINEERING A MAGNETOENZIMATIC MICROCARRIER SYSTEM TO DESIGN CHANNEL-LIKE TOPOGRAPHIES FOR FUTURE BONE REPAIR

Maria Cândida Mendes[P][1], Lúcia Isabel Santos[1], Ana Sofia Silva[1], João Filipe Mano[1]

[1] *CICECO-Aveiro Institute of Materials*

Corresponding author's email: mariamendes@ua.pt

To meet the growing demand for engineering relevant materials with a functional vasculature, the cliché of a multidisciplinary field was never so required as today. Inspired by biology, chemistry and engineering approaches, we herein propose the development of a magnetoenzymatic carrier system to sculpt a topographical patterning that resembles the native vasculature within laminarin hydrogels. For that, magnetic nanoparticles were prepared and entrapped into a poly-L-lactic acid (PLLA) shell for the development of the magnetic microcarrier system.[1][2] The enzyme laminarinase, which is able to degrade laminarin, was further immobilized in the PLLA shell via aminolysis and glutaraldehyde coupling reactions, respectively.[3] Physicochemical characterization of magnetite PLLA microparticles (m-PLLA) revealed a spherical particle shape and a size ranging from 15-40μm, which is consistent with the physiological size of small vessels. Additionally, XRD diffractograms confirmed the successful entrapment of the magnetite in the PLLA shell, and its magnetic properties demonstrated a superparamagnetic behaviour with a value of saturation magnetization of 3.7emu/g. The efficacy of enzyme-functionalized m-PLLA was assessed using the Bradford assay and its enzymatic activity by the 3,5-dinitrosalicylic acid (DNS) method. Regarding the sculpture process, the effect of magnetoenzymatic degradation on triggering channel-like topographies was assessed through confocal imaging. In the future, stem and endothelial cells will be seeded on the exposed construct, aiming to expedite a new therapeutic strategy towards bone repair. We acknowledge the FCT project “PROMENADE”-PTDC/BTM-MAT/29830/2017, the doctoral grant SFRH/BD/146740/2019 and the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (UID /CTM /50011/2013).

Keywords

magnetoenzymatic microcarrier ; topographical patterning; 3D hydrogels

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Abstract **#919**

THE SOURCE OF MULTIPOTENT MESENCHYMAL STROMAL CELLS AND XENO-FREE SUPPLEMENT IN DIFFERENTIATION MEDIUM DETERMINES THE EFFICIENCY OF INDUCED ADIPOGENESIS FOR ADIPOSE TISSUE ENGINEERING

Yuriy Petrenko[P][1], Eliska Vavrinova[1], Simona Stuchlikova[1], Milada Chudickova[1], Irena Vackova[1], Sarka Kubinova[1]
[1] *Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic*
Corresponding author's email: yuriy.petrenko@iem.cas.cz

Multipotent mesenchymal stromal cells (MSCs) are promising in adipose tissue engineering. However, little is known about the efficiency of adipogenic differentiation depending on the cell source. The clinical application of MSCs for adipose tissue engineering requires the use of xeno-free supplements, but their effect on adipogenic differentiation is not fully revealed. Here, we describe the comparative analysis of the efficiency of adipogenic differentiation of MSCs from human adipose tissue (AT) and Wharton's jelly (WJ) in media containing human derived supplements – human platelet lysate (PL), human serum albumin (HSA) or human serum (HS).
Human WJ and AT-MSCs were obtained according to ethical guidelines and expanded in the presence of 5% PL. Cells were differentiated in adipogenic lineage, using conventional inducers, different concentration of glucose (5 and 25 mM) and either FBS, PL or HSA as supplements (5%). The efficiency of adipogenic differentiation was assessed by Nile Red staining and quantification using Tecan Infinite 200 Pro reader.
The efficiency of adipogenic differentiation was remarkably higher in AT-MSCs compared to WJ-MSCs, in all studied conditions. The absence of medium supplement minimized the adipogenic differentiation of AT- and WJ-MSCs. The supplementation of differentiation medium by PL significantly lowered the efficiency of adipogenic differentiation of MSCs from both sources. However, the use of HSA or HS can be used as a xeno-free supplement, providing efficient adipogenic differentiation of MSCs for clinical applications. The MSC adipogenesis in xeno-free conditions was further confirmed in 3D collagen based scaffolds.
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Keywords
Adipogenic differentiation; xeno-free; adipose tissue engineering

Abstract **#920**

CONDUCTIVE ELASTOMER-BASED BIOELECTRONIC DEVICE FOR THE TARGETED DELIVERY OF CHEMOTHERAPY

Christopher A. R. Chapman$_P$[1], Shanila Fernandez Patel[1], Estelle A. Cuttaz[1], Catalina Vallejo Giraldo[1], Josef A. Goding[1], Rylie A. Green[1]

[1] *Imperial College London*

Corresponding author's email: c.chapman@imperial.ac.uk

Non-resectable glioblastoma multiforme is an untreatable brain cancer with treatment options limited to radiotherapy and systemic chemotherapy. Treatment with localised delivery of chemotherapy has seen limited clinical use due to diffusion limitations and increased intracranial pressure[1]. Ionic drug delivery has the potential to mitigate these challenges by delivering chemotherapy without the use of a liquid carrier[2]. This mode of delivery causes no increase in the volume to the surrounding tissue and therefore can improve bioavailability of chemotherapeutic drugs at the tumour.

Conductive elastomers (CE) consisting of a dispersion of a doped conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) in polyurethane[3] demonstrate promise as a unique platform for ionic drug delivery. To evaluate the efficacy of a CE-based platform, release profiles of both positively and negatively charged drug analogues were investigated. The voltage-driven release of the small molecule drug analogues methylene blue (MB) and fluorescein (FITC) were investigated over a range of applied voltages. Maximum increases in release were found to be a 5.4-fold increase at +0.5 V for MB and a 3-fold increase at -3 V for FITC.

Using these voltages, the release of charged chemotherapeutic molecules doxorubicin and cisplatin was quantified. Clinically relevant concentrations of both drugs ranging from 1 – 100 µM were achievable through voltage application. Experiments are underway to confirm the active anti-cancer effect of the device in vitro. This technology constitutes a significant step forward in bioelectronics-based cancer treatments and shows promise for ease of translation into clinical use to improve the outcome of patients with non-resectable GBM.

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Abstract **#921**

MAGNETIC CELL LABELLING WITH BIORESORBABLE NANOPARTICLES: AN ATTRACTIVE ADVANCED APPROACH TO GUIDE CELLS IN A 3D THYROID GLAND MODEL

Silvia Panseri[1], Monica Montesi[1], Giada Bassi[1], Elisabetta Campodoni[1], Margherita Montanari[1], Anna Tampieri[1], Monica Sandri[1]

[1] *Institute of Science and Technology for Ceramics - National Research Council of Italy*

Corresponding author's email: silvia.panseri@istec.cnr.it

Magnetic cell targeting represents a promising approach in medicine for several applications (e.g. cell therapy, tissue engineering). The goal is to guide the cells, in a precise way, in a 3D environment to achieve a better and most rapid organ/scaffold colonization, and/or loss less cells. Considering the importance of vascular network in reproducing predictive in vitro models such as thyroid organ, we focused our research in the design and the optimization of a protocol to obtain magnetic endothelial cells that can be finely guided and localized by an external magnetic field during the 3D printing process. For this aim we designed novel iron-doped hydroxyapatite nanoparticles (FeHA NPs), fully biocompatible and bioreasorbable nanoparticles, and we verified their behavior once internalized by the cells. In details human umbilical vascular endothelial cells (HUVECs) were culture with 100 µg/ml FeHA NPs, and commercial magnetic particles (SPIONs) were used as control. We successfully defined a magnetic labelling protocol. This protocol permits to obtain magnetic cells that can be moved by a static magnetic field application (450 mT). Magnetic HUVECs could be used to enhance the vascular network formation, in order to obtain an advanced 3D scaffold where thyroids cells could be seeded. In this way we could achieve a predictive thyroid model for the final screening of the endocrine disruptors.

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Abstract **#922**

IMPACT OF SEX-BASED DIFFERENCES ON CARTILAGE TISSUE ENGINEERING WITH CHONDROCYTES

Lianna R. Gangi[P][1], Hagar M. Kenawy[1], Aaron M. Stoker[2], James L. Cook[2], Nadeen O. Chahine[1], Gerard A. Ateshian[1], Clark T. Hung[1]

[1] *Columbia University*

[2] *University of Missouri*

Corresponding author's email: lrg2147@columbia.edu

The incidence of osteoarthritis (OA) is higher in women than in men. Sex as a variable is rarely considered in animal studies and in vitro experiments of OA[1-3]. There is a need to incorporate both male and female subjects in the development of cell-based, therapeutic strategies. We investigated the intrinsic differences of de novo extracellular matrix quality between engineered cartilage derived from male and female chondrocytes.

Articular cartilage was harvested from skeletally mature canine humeral heads. Cells were encapsulated in clinically-relevant agarose constructs and cultured in chondrogenic medium. Constructs were assessed for equilibrium modulus (EY) and biochemical content. Data were analyzed with mixed-effects analysis followed by Sidak's multiple comparisons test ($\alpha < 0.05$).

The sex of the animal did not statistically influence the day 42 EY values, glycosaminoglycan and collagen content of the engineered constructs. Both male and female constructs approached native EY and glycosaminoglycan levels, indicating that the culture procedure, which excluded sex hormones, could grow functional tissue irrespective of donor sex. Our findings may have implications on clinical translation, where the same culture regimen can be used to fabricate cartilage derived from male or female chondrocytes. Sex hormone supplementation to culture will be evaluated in the future and may reveal sex-specific differences in tissue development. Moreover, the impact of matching engineered cartilage with recipient sex on graft clinical performance remains to be elucidated with in vivo studies. Defining the importance of sex-based differences in cartilage growth may better direct the development of cell-based therapeutics for OA treatment.

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Abstract **#923**

ENGINEERING IN PRECISION MEDICINE

Ali Khademhosseini[P][1]

[1] *Terasaki Institute*

Corresponding author's email: khademh@terasaki.org

Engineered materials that integrate advances in polymer chemistry, nanotechnology, and biological sciences have the potential to create powerful medical therapies. Dr. Khademhosseini's group is interested in developing 'personalized' solutions that utilize micro- and nanoscale technolgoies to enable a range of therapies for organ failure, cardiovascular disease and cancer.In enabling this vision he works closely with clinicians (including interventional radiologists, cardiologists and surgeons). For example, he has developed numerous techniques in controlling the behavior of patient-derived cells to engineer artificial tissues and cell-based therapies. His group also aims to engineer tissue regenerative therapeutics using water-containing polymer networks called hydrogels that can regulate cell behavior. Specifically, he has developed photo-crosslinkable hybrid hydrogels that combine natural biomolecules with nanoparticles to regulate the chemical, biological, mechanical and electrical properties of gels. These functional scaffolds induce the differentiation of stem cells to desired cell types and direct the formation of vascularized heart or bone tissues. Since tissue function is highly dependent on architecture, he has also used microfabrication methods, such as microfluidics, photolithography, bioprinting, and molding, to regulate the architecture of these materials. He has employed these strategies to generate miniaturized tissues. To create tissue complexity, he has also developed directed assembly techniques to compile small tissue modules into larger constructs. It is anticipated that such approaches will lead to the development of next-generation regenerative therapeutics and biomedical devices.

Abstract **#924**

TISSUE ENGINEERED CARDIAC PATCHES FOR THE TREATMENT OF POST-MI HEART FAILURE USING NATURAL POLYMERS AND HUMAN IPSC-DERIVED CELLS

Annabelle TR Fricker[P][1], David A Gregory[1], Sian Harding[2], Ipsita Roy[1,2]

[1] *Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK*

[2] *National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, UK*

Corresponding author's email: atrfricker1@sheffield.ac.uk

Cardiovascular diseases (CVDs) are the leading cause of death worldwide and therefore pose a huge burden on healthcare systems. Myocardial infarction (MI) is one of the most fatal results of CVDs, as it can cause ultimate heart failure. Current available treatments, including coronary angiography, coronary angioplasty, or blood-thinning medication, are able to mitigate the MI symptoms but do not repair the damaged tissue. A final option is a heart transplant, however lack of organ donors and the subsequent lifelong reliance on immune-suppressant drugs reduces the popularity of this option. A potential solution to this issue is the use of a tissue engineered patch to deliver healthy cells to repopulate the area of infarct.

In this work we have developed a cardiac patch to replace the scar tissue developed post MI, using a family of bioresorbable and biocompatible polymers called Polyhydroxyalkanoates (PHAs)(1-4). Poly(3-hydroxyoctanoate-co-3-hydroxydecanoate), P(3HO-co-3HD), a medium chain length PHA, has been produced using a Pseudomonas species and thoroughly characterised. P(3HO-co-3HD) exhibited highly elastomeric properties, ideal for cardiac tissue engineering applications and a low melting temperature, ideal for 3D printing. Multimaterial 3D printed patches have been produced using P(3HO-co-3HD) including induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) encapsulated in a natural alginate hydrogel as an infill. In vitro cytotoxicity assays and imaging has been carried out on the complete patch.

Keywords

Polyhydroxyalkanoates; Alginate; Induced pluripotent stem cells

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Abstract **#925**

INTRAVENOUS INJECTION OF AUTOLOGOUS BONE MARROW-DERIVED MESENCHYMAL STEM CELLS ON THE GENE EXPRESSION AND PLASMA LEVEL OF CCL5 IN REFRACTORY RHEUMATOID ARTHRITIS

Mina Alavi[1,2], Jalil Tavakkol-Afshari[1], Zhaleh Shariati-Sarabi[3,4], Arezoo Gowhari Shabgah[5,6], Mohsen Ghoryani[7,8], Ali Ghasemi[9], Mojgan Mohammadi[2]

[1] *Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

[2] *Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

[3] *Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

[4] *Internal Medicine Department, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*

[5] *Student Research Committee, School of Medicine, Bam University of Medical Sciences, Bam, Iran*

[6] *School of Medicine, Bam University of Medical Sciences, Bam, Iran*

[7] *Department of Laboratory Sciences, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran*

[8] *Research Center of Advanced Technologies in Medicine, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran*

[9] *Department of Pediatric, Hematology and Oncology and Stem cell Transplantation, Mashhad University of Medical Sciences, Mashhad, Iran*

Corresponding author's email: mina.alavi91@gmail.com

Context: Rheumatoid arthritis (RA) is the most prevalent autoimmune disease, in which CCL2 and CCL5 are critically involved.

Aims: The objective was to evaluate the therapeutic effects of bone marrow-derived mesenchymal stem cells (MSCs) on the foregoing chemokines in RA patients.

Settings and Design: Thirteen RA patients were evaluated in terms of clinical manifestations, paraclinical factors, gene expression, and plasma levels of CCL2 and CCL5 prior to treatment and 1 and 6 months after intervention.

Subjects and Methods: Real-time-polymerase chain reaction and enzyme-linked immunosorbent assay were employed to assess the gene expression and plasma levels of CCL2 and CCL5 at different time points after MSC therapy. Statistical analysis was performed by SPSS 16 and Prism 7.

Results: The CCL2 gene expression had statistically significantly increased ($P = 0.034$), and its plasma level had insignificantly reduced after 1 month. Furthermore, the gene expression and plasma level of CCL5 had statistically significantly decreased ($P = 0.032$, $P < 0.001$). The CCL5 gene expression had statistically significantly increased after 6 months ($P = 0.001$) and its plasma level had insignificantly reduced.

Conclusions: The most significant inhibitory effects of MSC therapy on the gene expression and plasma level of CCL5 were observed at the end of 1 month. The differences between the gene expression and protein levels during the treatment might be related to microRNA effects or the insufficient number of MSC injection.

Keywords

chemokine CCL5; mesenchymal stem cells; rheumatoid arthritis

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Abstract **#928**

ASSESSING THE ELASTIC PROPERTIES AND CYTOCOMPATIBILITY OF DECELLULARISED ECM-DERIVED HYDROGELS FROM PORCINE LIVER

Elisa Tarsitano$_P$[1], Jing Yang[1], Lisa White[1], Andrew Bennett[2]

[1] *University of Nottingham School of Pharmacy*

[2] *University of Nottingham Medical school*

Corresponding author's email: Elisa.Tarsitano@nottingham.ac.uk

The extracellular matrix (ECM) comprises a number of large macromolecules that play an important role in cellular activities such as motility, differentiation and adhesion. The reproduction of a scaffold with comparable composition to the ECM is therefore highly desirable as it can provide a 3D environment which can replicate the liver architecture and biochemical complexity. One of the main challenges of producing such a scaffold is to find suitable replacement biomaterials that best mimic, not only the extra cellular matrix but also the biological organisation of the tissue. Hydrogels have been explored due to their biocompatibility, degradability and having similar mechanical properties to ECM. Adult progenitor liver cells have been identified as bipotent cells capable of differentiating to hepatocytes and cholangiocytes. Given the right cues and three-dimensional environment, progenitor liver cells can be differentiated into functional hepatocyte-like cells.

In this study, porcine livers were decellularised using an enzymatic and detergent washing process. Decellularised livers were digested and solubilised to form hydrogels. DNA and glycosaminoglycan content were assessed, and hydrogel mechanical properties characterised.

Human progenitor liver cells were encapsulated in porcine ECM-derived hydrogels to create in-vitro liver scaffolds. The cells remained viable for at least 10 days and showed increased proliferation when encapsulated in lower concentrations of ECM. Efficiency of organoids formation was comparable with the commercial matrix BME2.

This novel approach can be utilised to produce a 3D environment that closely resemble the liver niche to culture progenitor liver cells and differentiate them into functional hepatocyte-like cells.

Keywords

Organoids; Hydrogels; Extracellular matrix

Abstract **#929**

DEVELOPMENT OF A POLYESTER BASED VASCULAR EQUIVALENT WITH HUMAN MESENCHYMAL STEM CELL-DERIVED SMOOTH MUSCLE CELLS AND ENDOTHELIAL CELLS

Gozde Ervin Kolep[1], Halime Kenar[2,3], Vasif Hasirci[1,2,3], Deniz Yucel[1,3,4]

[1] *Department of Medical Biotechnology, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey*

[2] *Department of Medical Engineering, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey*

[3] *BIOMATEN, METU, Center of Excellence in Biomaterials and Tissue Engineering, Middle East Technical University, Ankara,Turkey.*

[4] *Department of Histology and Embryology, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey*

Corresponding author's email: gozdeervin@gmail.com

Tissue engineering is a promising approach for the treatment of damaged blood vessels. The aim of this study was to develop a small-diameter tissue engineered arterial equivalent by mimicking layers of arterial wall. The scaffold was composed of porous tubular film prepared by dip coating, circumferentially aligned electrospun fibers collected over the film layer, and random electrospun fibrous mat which was wrapped over the aligned fibrous layer. The lumen of tubular scaffold was seeded with human umbilical vein endothelial cells (HUVECs), while human Wharton's Jelly mesenchymal stem cells (WJ MSCs) were seeded onto the circumferentially aligned fibrous mat. Proliferation and organization of the cells on the scaffold were investigated with MTS and Phalloidin-DAPI, respectively. WJ MSCs were differentiated into smooth muscle cells (SMCs) on the scaffold. The expression of specific markers by HUVECs and SMCs was investigated by immunocytochemistry. The tubular scaffold having ca. 3 mm inner lumen diameter was obtained successfully. HUVECs were evenly distributed on inner side of the porous film, while WJ MSCs were oriented on the circumferentially aligned fibers along the fiber axis. It was observed that WJ MSC-derived SMCs and HUVECs preserved their cell morphology and cell specific protein expression on the scaffolds. The results indicate that the tissue engineered vascular substitute developed would be a potential therapeutic approach in regenerative medicine for the treatment of arterial diseases.

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Abstract **#930**

DEVELOPMENT OF A 3D MICROENVIRONMENT FOR ENGINEERING OF GLIOBLASTOMA BRAIN TUMOR

Jasmina Stojkovska[1,2], Tijana Stankovic[3], Miodrag Dragoj[3], Jelena Petrovic[1,2], Mia Radonjic[1,2], Milica Pesic[3], Bojana Obradovic[1]

[1] *Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

[2] *Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia*

[3] *Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade*

Corresponding author's email: jstojkovska@tmf.bg.ac.rs

The aim of this work was to develop a 3D microenvironment for glioblastoma brain tumor engineering based on alginate hydrogels as a matrix for cell immobilization followed by cultivation in a biomimetic perfusion bioreactor. Alginate microfibers with immobilized cells were obtained by a simple extrusion technique. We have examined the influence of the needle diameter (22G - 28G), cell density in alginate solution (1 x 106 - 8 x 106 cells/ml) and different cancer cell lines (rat C6 and human U251 and U87) on cell immobilization efficiency and viability. The best alginate microfibers (500 µm in diameter) with all immobilized cells were obtained by applying a 25G needle with a minimal cell density of 4 x 106 cells/ml. The obtained microfibers with immobilized cells (C6 and U87) were cultivated in a perfusion bioreactor at the continuous medium flowrate in the range 0.05-0.30 ml/min over short- and long-term cultivation periods. The results have shown that the flowrate of 0.30 ml/min, corresponding to the superficial velocity of 100 µm/s, in combination with the C6 cell density of 8 x 106 cells/ml in short-term studies yielded higher cell viabilities and proliferation as compared to the control static culture. In addition, U87 cells immobilized in alginate microfibers at the density of 4 x 106 cells/ml after long-term cultivation at the medium flowrate of 0.05 ml/min (superficial velocity of 15 µm/s) stayed viable.

The overall results have shown potentials of the applied approach for tumor engineering provided optimization of cultivation conditions for each cell type.

Abstract **#931**

CLIMBING THE VASCULAR TREE IN TISSUE ENGINEERING

Debby Gawlitta[P][1,2]

[1] *University Medical Center Utrecht*

[2] *Regenerative Medicine Center Utrecht*

Corresponding author's email: d.gawlitta@umcutrecht.nl

Nowadays, de novo vasculogenesis and subsequent angiogenesis in small engineered tissues is common practice. Also, channeling the shape of the vascular tree into bioinks and decorating them with endothelial cells appears a well-trodden path. As a consequence, the engineering of interconnected vascular structures that can be readily perfused and implanted seems not too far away from reality.

Contrarily, when taking a closer look at current achievements and how to transform them to clinically implantable and perfusable cm-scale tissues, there are still numerous challenges to be met. These include functional interconnection of multi-scale vasculature, creating and sustaining the surrounding target tissue, and implantation in and anastomosis to a recipient.

Complex in vitro models are presented that show interconnection of capillary-like networks in hydrogels with endothelialized channels.(1) The presence of another viable tissue, in which the vasculature is embedded, requires finetuning of culture regimes to accommodate multilineage cell differentiation and maintenance. The hydrogel-based models cannot be sutured to the existing vasculature of a recipient. An alternative is to recapitulate the intricate multilayer structure of small diameter blood vessels by electrospinning bilayered vascular grafts that are suturable in a small animal model. The heterotypic culture of endothelial cells and smooth muscle cells on such a graft requires multifaceted culture regimes.(2) Next steps require ingenuity of the TERMIS community to provide suitable biomaterials for biofabrication of tissues accommodating a vascular tree, increased control of capillary-to-vessel connection, intricate culture regimes, and animal models that enable evaluation of vascularized specialized tissues in their natural niche.

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Abstract **#933**

NANOFIBROUS NETWORKS WITHIN LIQUEFIED-CORE SYSTEMS FOR TISSUE ENGINEERING APPLICATIONS

Miguel Simões[P][1,2]

[1] *University of Aveiro*

[2] *Aveiro Institute of Materials- CICECO*

Corresponding author's email: miguelgsimoes@ua.pt

As the field of tissue engineering and regenerative medicine (TERM) evolved, new tissue repair technologies have emerged. An alternative to conventional hydrogels with fixed geometries, consists of Liquefied Multi-layered Capsules (LMC)[1]. Comprised of a layer-by-layer membrane, a liquefied alginate core containing cells and poly(L-lactic)acid (PLLA) nanofibers, LMC allow free movement of cells across the 3D construct, while maximizing the diffusion of essential molecules. Nanofibers are of utmost interest given its function as an anchoring and assembly 3D system, mimicking the fibrous microenvironment of the extracellular matrix found in vivo. Considering their higher surface area and aspect ratio, compared to microparticles, nanofibers may represent superior cell carrier systems, and aid in cell orientation. To test this hypothesis, PLLA nanofibers were co-encapsulated with stromal cells, under dynamic conditions, and compared with LMC containing only cells (control).

Results show that, after fragmentation of electrospun mats, nanofibers ranged between 5 and 50µm and 1 and 2µm, in length and diameter respectively. Upon encapsulation, the inclusion of nanofibers promoted the formation of cell agglomerates within the LMC, containing biomimetic fibrous networks. Cells shown to remain viable, throughout 14 days of culture, but also metabolically active, in both conditions. Together with dynamic stimulus, a closer representation of in vivo conditions is accomplished, thereby possibly serving as hybrid devices implantable by minimally invasive procedures for TERM applications.

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Keywords

nanofibers; liquified systems; tissue engineering

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Abstract **#935**

DEVELOPMENT OF SCAFFOLD BASED PLATFORMS FOR LIVER AND KIDNEY RELATED DISEASES

Anthony Callanan[P][1]

[1] *Institute for Bioengineering, School of Engineering, University of Edinburgh*

Corresponding author's email: anthony.callanan@ed.ac.uk

Chronic kidney and liver disease are major global health problems, with drastic shortages in organs available for clinical transplant limiting patient treatment options. Tissue engineering is a rapidly accelerating field; recent advances using induced human pluripotent stem cells to create functional organoid structures for human organs hold great promise for patients (1, 2). To further advance these and address their limitations, our research is focussing on optimising the cellular microenvironment.

We have developed polymer-based scaffolds alongside 3D printed bioreactors as conditioning tools for cells and organoids, providing a highly controlled and customizable microenvironments within which to influence cell differentiation, survival and function. These bioreactors were optimised using an in silico approach in their design allowing control over the applied stimulus. Our results highlight the complexity of the cellular microenvironment shear stress and strain stimuli, while demonstrating how scaffold design has inherent functions in controlling biological response.

The findings highlight the need for further consideration into understanding and controlling niche cellular microenvironments. Advanced platforms such as ours are emergent innovative tools which will play a major role into treatment optimisation strategies in chronic diseases.

ACKNOWLEDGEMENTS: This work is funded by an EPSRC doctoral training partnership studentship, UK Regenerative Medicine Platform II [RMPII] grant MR/L022974/1 and MRC grant CCBN MR/L012766/1. This work is enabled by the sacrifice of UK organ donors and their relatives, with the assistance of NHS Organ Donation and Transplant.

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Abstract **#936**

SPONTANEOUS 3D-MICROPATTERNING OF BMP-2 IN SELF-ASSEMBLING NANOCLAY GELS FOR BONE TISSUE REGENERATION

Roxanna Ramnarine Sanchez$_P$[1], Nicholas Evans[1], Richard Oreffo[1], Jonathan Dawson[1]

[1] *University of Southampton*

Corresponding author's email: rsrs1v19@soton.ac.uk

INTRODUCTION: Emulating the three-dimensional (3D) organization of biochemical cues present in cellular-microenvironments is likely to be key in developing biomaterials able to promote regeneration. However achieving stable structures incorporating 3D-micropatterning of biochemical cues that combine high cell-level resolution at clinical scale is challenging1. Nanoclay-gels have established potential in TE owing their capacity to sequester proteins for sustained/localised bioactivity2. The current study reports a method to self-assemble nanoclay-gels with spontaneous 3D-protein micropatterning to deliver localized/stable niches for bone regeneration.

METHODS: Clay suspensions (Laponite®) were added to solutions containing biomolecules present in blood plasma to initiate a diffusion-reaction mediated self-assembly process. Assembled structures were tested for their ability to pattern various fluorescently-labelled proteins and to localize the bioactivity of bone morphogenetic protein (BMP-2). The structures were analysed using fluorescent, polarized-light and electron microscopy, and functionality assessed through a 28-day murine subcutaneous bone induction assay.

RESULTS: Assembled structures provided a template for 3D-protein micropatterning, and spatial localization could be controlled down to a resolution of ~20µm by changing the assembly parameters, including concentration, ionic strength, incubation time and temperature. Furthermore, assembly of structures at scale with a range of dimensions (0.2-1mm) and shapes (droplets, cylinders, strings) was possible. Finally, the in vivo study indicated that localized BMP-2 was able to precisely template bone formation within the gel structure.

DISCUSSION & CONCLUSIONS: This study reveals the potential to harness interactions between clay-nanoparticles and biomolecules to design scaffolds with biochemical gradients, dimensions and shapes for bone with clinical relevance.

Keywords

Hydrogels; Drug delivery; Bone regeneration

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Abstract **#937**

EXTRACELLULAR MATRIX ENGINEERED FROM HIPSCS ENHANCES THE BONE REGENERATION POTENTIAL OF AGED HUMAN BONE MARROW STROMAL CELLS

Dominik Hanetseder[P][1], Tina Levstek[1], Heinz Redl[1], Darja Marolt Presen[1]
[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology*
Corresponding author's email: dominik.hanetseder@trauma.lbg.ac.at

Background and aim: Regeneration of bone defects in elderly patients is limited due to the decreased function of bone-forming cells and compromised tissue physiology. The aim of our project was to investigate whether extracellular matrix (ECM) engineered from human induced pluripotent stem cells (hiPSCs) can enhance the bone regeneration potential of aged human bone marrow stromal cells (hBMSCs).
Methods: ECM was engineered from hiPSC-derived mesenchymal-like progenitors (hiPSC-MPs), as well as young (<30 years) and aged (>70 years) hBMSCs. ECM structure and composition were characterized before and after decellularization using immunofluorescent stains and biochemical assays. Three hBMSC strains of different ages were cultured on engineered ECMs, and their growth and differentiation responses were compared to tissue culture plastic, as well as to collagen- and fibronectin-coated plates.
Results: Decellularized ECMs contained collagens type I and IV, fibronectin, laminin and < 5% residual DNA. Cultivation of young and aged hBMSCs on the hiPSC-ECM in osteogenic medium significantly increased hBMSC growth and osteogenic differentiation compared to tissue culture plastic controls and single protein substrates. In aged BMSCs, matrix mineralization was only detected in ECM cultures in osteogenic medium. ECMs engineered from hiPSC-MPs and hBMSCs of different ages exhibited similar structure, composition and potential to enhance osteogenic responses in aged BMSCs.
Conclusions: Our studies suggest that aged BMSC regenerative capacity can be enhanced by culture on hiPSC-engineered ECM. In particular, we showed that engineered ECM outperforms single-protein substrates. Tissue engineering strategies employing engineered ECM materials could thus potentially enhance bone regeneration in elderly patients.

Abstract **#939**

FIBRILLAR MESH HYDROGELS FOR LIVING BIONIC DEVICES

Catalina Vallejo-Giraldo[P][1], Martina Genta[1], Roberto Portillo-Lara[1], Josef Goding[1], Rylie Green[1]

[1] Imperial College London

Corresponding author's email: cata.vallejo@gmail.com

The use of cell laden hydrogel matrices as soft tissue interfaces has led to the concept of "living electrodes", which refers to bionic devices with a functional neural cell component1. The "living" layer is proposed to actively drive the integration of the device into the endogenous tissue1. To achieve this, a cell supportive network with optimal structural stability and microarchitecture must be formed to support cell viability and guide function. The aim of this study was to investigate the use of a biological polymer, collagen, for providing topographical cues within a synthetic poly(vinyl alcohol)-tyramine (PVA-Tyr) hydrogel. Collagen type I was hypothesized to promote cellular migration and infiltration to encapsulated neuroprogenitor cells, facilitating the development of 3D neural networks. PVA-Tyr-Col hydrogels were synthesized using different mass ratios of collagen to PVA-Tyr (1:1 to 1.5:1) and the physical and mechanical properties of the resulting scaffolds were evaluated using AFM. Hydrogel cytocompatibility was tested in vitro using 3D primary neuron culture. Immunological staining was carried out using monoclonal anti-β-tubulin and anti-GFAP antibodies, to evaluate the growth of neurons and astrocytes, respectively. In addition, the formation of functional neural networks was assessed via calcium imaging and Piezo1 expression. PVA-Tyr-Col hydrogels were found to support the growth of both neuronal and astrocytic cell populations with improved viability, compared to PVA-Tyr-gelatin hydrogels which contain similar bioactive peptide sequences but lack the topographical cues imparted by the whole protein. PVA-Tyr Col hydrogels provide a promising platform for the development of living interfaces for stimulating bioelectronic devices.

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Abstract **#940**

THE SYNERGY AND COMPETITION BETWEEN PHYSICAL AND BIOLOGICAL EFFECTS IN SHOCKWAVE THERAPY

Cyrill Slezak$_P$[1,2], Paul Slezak[2], Julia Böhm[2,3], Sophie Hermann[2,3]

[1] *Utah Valley University*

[2] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology*

[3] *Technical University of Vienna*

Corresponding author's email: cyrill.slezak@gmail.com

The application of extracorporeal shock wave therapy (ESWT) on tissues results in well establish therapeutic and regenerative benefits to the patients. Through mechanotransduction of the incoming pressure pulse, a cascade of biochemical responses is triggered within the cells. Simultaneously, there is a non-linear response in the physical bulk properties of the targeted structures. Either one of those processes may be both beneficial or detrimental for the treatment outcome. We present findings on the efficacy of treating lung tissue using low energy shockwaves which is normally considered a counter-indication for the therapy. Potential attenuated proinflammatory cytokine expression and regenerative benefits have to be seen in the light of potentially destructive tensile forces imparted on the lungs resulting in pulmonary capillary hemorrhages. Based on water bath reference measurements, computational simulations, and in-vivo experiments we provide a comprehensive overview of competing considerations in moving towards a clinical use of ESWT in the potential treatment of a wide range of new indications.

Abstract **#941**

BIOINSPIRED OXIDATION RESISTANT SLIDING RING POLYROTAXANE-CATECHOL HYDROGELS FOR TISSUE ENGINEERING

María Isabel Rial-Hermida[P][1,2], Dora C. S. Costa[1], Lan Jiang[3], Carmen Alvarez-Lorenzo[2], Kohzo Ito[3], Joao F. Mano[1]

[1] CICECO Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193, Aveiro, Portugal.

[2] Departamento de Farmacia, Farmacoloxía e Tecnoloxía Farmacéutica. Facultade de Farmacia. Universidade de Santiago de Compostela, 15872, Santiago de Compostela, Spain.

[3] Department of Advanced Materials Science, Graduate School of Frontier Sciences. The University of Tokyo, Tokyo, Japan.

Corresponding author's email: Mariaisabel.rial@usc.es

Introduction: Hydrogels have been widely used in tissue engineering to accommodate the culture of cells. A novel class of catechol-functionalized polymers could lead to hydrogels with improved mechanical properties by designing reversible crosslinkings using metallic coordination. This reversible coordination is depending on pH and promoted at slightly basic pH(1), as long as the oxidation of the catechol group be avoided(2). On other hand, supramolecular entities namely polyrotaxanes (PR) could form hydrogels with similar mechanical properties of cartilage, with the possibility of promote the differentiation of pluripotential stem cells due to the capacity of the cyclodextrin to slide trough the PEG chain and be loaded with active agents(3). However, PR functionalized with catechol residues is not explored yet.

Methods and Results: PRs were functionalized by EDC/NHS coupling reaction(4) with a novel nitrocatechol synthetized in our lab, following a modified methodology described to avoid the quickly oxidation of entities containing catechol(5). Functionalization was confirmed by H1NMR. Functionalized supramolecular entities were then dispersed in aqueous media, and hydrogels were prepared(2).The elastic and the viscous moduli and the self-healing ability of formed hydrogels were evaluated. Cytocompatibility of hydrogels was ensured with SAOS-2.

Conclusions: A biocompatible cutting-edge reversible crosslinked hydrogel was developed, taking advantage of a functionalized sliding ring polyrotaxanes with nitrocatechol, able to avoid the issues of oxidation. This completely novel combination results in hydrogels with capacity to distribute the typical stresses that suffer the biomaterials, for example in cartilage substitution, along the entire volume of the hydrogel, trough reversible free movements of cyclodextrins.

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Abstract **#942**

TOPOGRAPHICAL PATTERN TRANSFER TO POLYACRYLAMIDE GELS FOR CARDIAC CELL ALIGNMENT

Bita Behziz$_P$[1], Lian Wong[2], Rachel Hatano[2], Jose Zamora[3], Kara E McCloskey[2,4]

[1] *School of Engineering, University of California Berkeley*

[2] *Graduate Program in Biological Engineering and Small-scale Technologies (BEST), University of California Merced*

[3] *School of Natural Sciences, University of California Merced*

[4] *School of Engineering, University of California Merced*

Corresponding author's email: bita.behziz@gmail.com

During a myocardial infarction or a heart attack, cardiomyocytes (CM) are deprived from oxygen, leading to death with almost no regeneration. Tissue engineering holds promise towards developing solutions to produce more efficient diagnostic and drug discovery platforms, patient-specific organ transfer, and models for basic science research. Embryonic stem cells and induced pluripotent stem (iPS) cells can differentiate into all cell types, making them a valuable cell source for applications in tissue engineering. Moreover, because iPS cells can be made patient-specific, their derivatives could also eliminate the need for immunosuppressive therapies. In order to direct the cell fate of human iPS to CM cell lineage, mechanical forces have been shown to play an important role such as mechanical strain, topography-guidance, and substrate stiffness. Our goal for these studies was to culture iPS-derived CM on wrinkled microchips and examined alignment on various surfaces. We used a cost-effective shrink-based fabrication method to create the wrinkle topography for guiding cell alignment on physiological stiffness. The results show that the pattern can be retained in PDMS as well as lower stiffness polyacrylamide (PA) hydrogels, but the CM preferentially adhered to the flat glass rather than the wrinkled PA. Future work plans to optimize the cell seeding density, extracellular matrix proteins and the linker's concentration in order to increase the adhesion of CM on PA.

Abstract **#944**

TOUGH ANTIBACTERIAL HYDROGELS AS TISSUE ADHESIVES FOR WOUND HEALING

Yumin Yang$_P$[1], Jue Ling[1]

[1] *Key Laboratory of Neuroregeneration, Nantong University*

Corresponding author's email: jl2016@ntu.edu.cn

INTRODUCTION: Current shortcoming in tissue adhesives commonly used in clinical practice was based on their poor tissue compatibility and/or weak tissue adhesion.1,2

METHODS: Evaluation of tissue adhesion of the hydrogels, in vitro and in vivo cytocompatibility and in vivo wound healing.

RESULTS: Our biocompatible hydrogels comprising sodium alginate, chitosan, and polyacrylamide exhibited good cytocompatibility, antibacterial activity, and excellent tissue adhesion.

DISCUSSION & CONCLUSIONS: We developed biocompatible hydrogels comprising sodium alginate, chitosan, and polyacrylamide and exhibiting good cytocompatibility, antibacterial activity, and excellent tissue adhesion. The hydrogels exhibited significantly enhanced mechanical properties following the addition of polyacrylamide, which synergistically promoted their enhanced adhesion. Moreover, results showed that the hydrogels exhibited good biocompatibility and antibacterial activity and promoted wound recovery during use as wound dressings.

Acknowledgements:We thank the financial support from National Science Foundation of China.

Keywords: Bioadhesives, Biomaterials

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Abstract **#945**

FREESTANDING SELF-HEALING COATING TOWARDS TISSUE REPAIR

Jue Ling$_P$[1]

[1] *Key Laboratory of Neuroregeneration, Nantong University*

Corresponding author's email: jl2016@ntu.edu.cn

INTRODUCTION:Self-healing materials have received intense attention because of their excellent ability of restoring their properties and shapes after automatically repairing their damaging sites without additional adhesives, whose ultimate aim is to improve the resilience of safety-critical components.[1]

METHODS:SF-βCD or HA-AD were alternately deposited on a glass surface to fabricate the self-healing (SF-βCD/HA-AD)*n coatings. Cytocompatibility evaluation of the coating by MTT.

RESULTS:The freestanding and self-healing (SF-βCD/HA-AD)*n coating based on host-guest interactions by layer-by-layer (LbL) self-assembly. This coating could easily and repeatedly heal external mechanical damage and be pulled of the glass substrate. Antibacterial and cytocompatibility evaluation illuminate that the coatings have an admirable antibacterial activity and cellular bioactivity.

DISCUSSION & CONCLUSIONS:Based on host-guest interaction, a freestanding self-healing coating with excellent biocompatibility and promising antibacterial activity was prepared by LbL assembly and we believe that the (HAD/SCD)n coatings will have great potential in tissue engineering.

Keywords: Biomaterials, Polymers - natural / synthetic / responsive

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Abstract **#946**

MODELLING A CELLULAR ELECTRIC MICROENVIRONMENT WITH THE FINITE ELEMENT METHOD

Miruna Verdes[P][1], Catherine Disney[1], Chinnawich Phamornnak[1], Lee Margetts[2], Sarah Cartmell[1]

[1] *Department of Materials, Faculty of Science and Engineering, The University of Manchester, Manchester, UK*

[2] *Department of Mechanical, Aerospace and Civil Engineering, The University of Manchester, Manchester, UK*

Corresponding author's email: miruna.verdes@postgrad.manchester.ac.uk

Electrical stimulation (ES) is a noninvasive potent tissue engineering tool whose mechanisms are yet to be fully understood. Despite promising experimental results, it is still difficult to optimize stimulation protocols due to the translational limitations between in vitro and in vivo. Stimulation devices as well as cellular microenvironment differ significantly between the two layouts. To bridge this gap, we used in silico finite element method (FEM) models to investigate the cell detectable signals, such as charge and current density, present in the microenvironment. Two types of scaffolds were modelled; either artificial, i.e. Poly(3,4-ethylenedioxythiophene) poly(styrenesulfonate) (PEDOT:PSS) coated silk fibers, or natural extracellular matrix (ECM) made of collagen fibers and interstitial fluid. Furthermore, our simulations show how fiber alignment to the electric field (EF) determines electrical microenvironment in nanofibrous electroconductive scaffolds. This observation can be explored to investigate cellular mechanisms of response to EF and design of scaffolds with complex fibrous networks for functional tissue engineering.

Keywords

Electrical stimulation; Finite element modelling; Cellular microenvironment

Abstract **#948**

CHITOOLIGOSACCHARIDES-INDUCED FIBROBLAST EXOSMES FACILITATE AXON GROWTH IN PERIPHERAL NERVE REGENERATION

Cheng Sun[P][1], Xin Tang[1], Yumin Yang[1]

[1] *Key Laboratory for Neuroregeneration of Jiangsu Province and Ministry of Education, Co-innovation Center of Neuroregenetation, Jiangsu Clinical Medicine Center of Tissue Engineering and Nerve Injury Repair, Nantong University, 19 Qixiu Road, Nantong, Jiangsu 226001, China*

Corresponding author's email: suncheng1975@ntu.edu.cn

Chitosan-based artificial nerve scaffolds have been identified as suitable products for peripheral nerve defects in clinical trials. In our previous study, chitosan degradation products, chitooligosaccharides (COS), could facilitate peripheral nerve regeneration by constructing macrophage-constructed microenvironments.

Fibroblast (Fbs) and Schwan cells (SCs) are main components of peripheral nerves. Exosomes carry functional miRNA and protein to operate their biological properties. In the present study, we observed that COS enhances exosome secretion from Fbs, which markedly promotes neurite outgrowth and nerve regeneration.

By using iTRAQ, protein profiles in the proximal nerves treated with COS were analyzed. COS promotes secretion of exosomes from Fbs and sequencing data revealed that miR-132-5p was decreased. Exosomes were prepared from Fbs with a commercial kit. RNA expression profiles in exosomes were analyzed by RNA sequencing. COS-induced exosomes remarkably stimulated axon growth in cultured DRG explants and injured sciatic nerves. Neurite outgrowth was evaluated by neurite length in cultured DRG explants. Nerve regeneration in vivo was assayed by behavioral performances, electrophysiological tests and morphological analysis. Inhibition of miR-132-5p also induced similar results in axon growth. Camkk1 is a target of miR-132-5p for transducing its promotion effect on axon growth.

Our present study further revealed a new aspect of COS in nerve regeneration, i.e. COS-induced exosomes from Fbs accelerate axon growth and the axis of miR132-5p/Cammk1 is responsible for this beneficial effect. Overall, our present data, together with our previous work, provide a strong theoretical basis for application of chitosan based biomaterials in peripheral nerve regeneration.

Abstract **#949**

IN VITRO MODELS OF OSTEOARTHRITIS INSPIRED BY DEVELOPMENTAL BIOLOGY

Laura Dönges[P][1], Alessandro Pirosa[1], Ivan Martin[1], Andrea Barbero[1]

[1] *Department of Biomedicine, University Hospital Basel, University of Basel, Switzerland*

Corresponding author's email: laura.doenges@usb.ch

Osteoarthritis (OA) is the most common degenerative joint disease worldwide and treatment options are limited to palliative care or, at the end stage of the disease, to total joint-replacement therapy. During OA progression, articular chondrocytes acquire hypertrophic traits followed by full remodelling of the cartilage into bone. Recent cartilage developmental biology studies showed that endogenous inhibition of bone morphogenetic protein (BMP) plays a central role in insulating articular stable cartilage from transient cartilage, which will be vascularized and undergo endochondral ossification. We hypothesize that by applying developmental biology concepts in vitro, BMP signalling inhibition can be applied to counteract hypertrophic differentiation of OA chondrocytes. To test this, human bone-marrow derived mesenchymal stromal cells were differentiated in vitro towards the chondrogenic lineage using a 3D high-throughput micro-aggregate culture system. After the chondrogenic phase, the generated micro-aggregates were exposed to inflammatory factors highly present in an OA joint (IL1, IL6 and TNF). At the end of such OA inducing phase, we showed a significant decrease of the expression of hyaline cartilage genes (COL-II and ACAN) and a significant increase of expression of the hypertrophic (MMP-13 and BMP2) and pro-inflammatory (IL6 and IL8) genes. Moreover, glycosaminoglycan content decreased in response to inflammation, whereas interleukin-8 and BMP2 secretion increased. We thus demonstrated that our model well recapitulates key features of OA cartilage. We are now using this model to investigate whether treatment by BMP type I receptor kinase inhibitors counteracts the evolution of such OA features as well as modulate angiogenic properties.

Abstract **#950**

HIGHLY BRANCHED POLY(B-AMINO ESTER)S AS A NEW GENERATION OF NON-RIVAL GENE DELIVERY VECTOR

Dezhong Zhou[P][1], Chenfei Wang[1]

[1] *Xi'an Jiaotong University*

Corresponding author's email: dezhong.zhou@xjtu.edu.cn

Since the first development of poly(β-amino ester)s in 2000, more than 2350 poly(β-amino ester)s have been synthesized and screened for gene transfection, and the most efficient candidate can even rival adenovirus in stem cells. However, until 2015, all the poly(β-amino ester)s developed had been linear structured. Herein, we have developed a highly controllable and flexible "A2+B3+C2" Michael addition strategy to synthesize highly branched poly(β-amino ester)s. The "A2+B3+C2" strategy not only effectively delays the gelation but also endows the synthesized polymers with a 3D topology with multiple terminal groups. In vitro gene transfection studies over 12 different cell types including stem cells and primary cells show that the gene transfection efficiency of highly branched poly(β-amino ester)s is up to 8521-fold higher than their corresponding linear counterparts. Highly branched poly(β-amino ester)s can also effectively deliver nerve growth factor (NGF) encoding DNA to PC12 cells to promote the outgrowth of neurites. In vivo, highly branched poly(β-amino ester)s can effectively deliver collagen VII encoding gene COL7A1 to fibroblasts and keratinocytes to restore the expression of collagen VII, which accelerates the wound healing of recessive dystrophic epidermolysis bullosa. Cytotoxicity studies further show that highly branched poly(β-amino ester)s can preserve high cell viability in diverse cell types and induce negligible inflammation in vivo. All these results highlight the great potential of highly branched poly(β-amino ester)s in non-viral gene therapy, in terms of high transfection efficiency and low cytotoxicity.

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Abstract **#951**

PREPARATION OF POLY (Ɛ-CAPROLACTONE-RAN-LACTIDE) SCAFFOLD USING LOW TEMPERATURE 3D PRINTING AND ITS PRINTABILITY TEST

Joon Yeong Park[1], Moon Suk Kim[1]
[1] *Ajou University*
Corresponding author's email: pjy16@ajou.ac.kr

3D printing has a method of manufacturing a complex and elaborate structure by dividing it into a certain thickness and stacking it layer by layer. Compared to other manufacturing methods, it has the advantage of being able to easily manufacture complex shapes. In the case of most 3D printing inks used a material that melts at a high temperature of 180 ℃ or higher is used, which has a disadvantage in that it is not possible to apply a temperature sensitive bioactive material. In this work, poly(ε-caprolactone-co-lactide) 3D printing ink was prepared by controlling the ratio of caprolactone and lactide monomer. The ratio of lactide to caprolactone was gradually increased to have a melting temperature of 40 to 60 ℃. We manufactured 3D printing scaffold at 110 ℃. We measured line width and porosity using Scanning electron microscope and stereomicroscope images and confirmed by comparing the modeled theoretical results. Under the same conditions, we confirmed that the higher the melting point, the more stable the shape was maintained. In the same material, the printing speed, temperature, and pressure were controlled to confirm appropriate printability. In conclusion, we manufactured 3D printing inks capable of printing at low temperatures compared to the existing printing methods and verified optimal printability. Also, we developed a 3D printing inks have potential using with thermal-sensitive materials.

Keywords
printability; polyester; melting point

Abstract **#952**

PREPARATION AND EVALUATION OF ADAPALENE EMULSION CONTAINING POLYCAPROLACTONE HAVING FUNCTIONAL GROUPS FOR IMPROVED TREATMENT OF SKIN DISEASES

Yun Bae Ji[P][1], Hye Yun Lee[1], Gi Ru Shin[1], Moon Suk Kim[1]

[1] *Ajou university*

Corresponding author's email: jyb26267@ajou.ac.kr

Polycaprolactone has the advantage of having adequate biocompatibility and biodegradability. However, it is difficult to impart additional functions for efficient disease treatment due to the absence of functional groups and strong hydrophobicity. In this study, an emulsion formulation containing polycaprolactone with an amine group (MCL-NH2) was developed to improve skin permeability through electrostatic interactions and applied together with adapalene (AD) to evaluate the effect of treating skin diseases. First, we manufactured polycaprolactone with a benzyloxy group (MCL-OBn) were by ring opening polymerization. After that, MCL-NH2 and AD-emulsion prepared through additional reactions. We confirmed through the dynamic light scattering (DLS) measurement that the larger the number of amine groups of MCL-NH2, the smaller the size of the emulsion particles due to electrostatic interaction with AD. Through stability evaluation, it was confirmed that the emulsion formulation was maintained for a time during which the drug in the emulsion could be sufficiently absorbed into the skin. Based on the above experimental results, we conducted in vitro and in vivo experiments, and proved that the anti-inflammatory and acne treatment effects are more effectively improved by the electrostatic interaction of the AD-emulsion with MCL-NH2. As a result, we confirmed that by manufacturing a polycaprolactone having a functional group, it is possible to impart the function of skin permeability improvement and drug-controlled release. In addition, it is expected to be a promising material that can further increase the treatment efficiency of various diseases and can be useful in tissue engineering and biomedical fields.

Keywords

polycaprolactone; emulsion; electrostatic interaction

Abstract **#953**

PREPARATION OF ANTICANCER DRUG-CONTAINING INTRATUMORALLY INJECTABLE HYALURONIC ACID HYDROGEL

Hyeon Jin Ju[P][1], Min Ju Kim[1], Gi Ru Shin[1], Moon Suk Kim[1]

[1] *Ajou University*

Corresponding author's email: tssos@ajou.ac.kr

The first method used to treat cancer is a surgical method in which the cancer tissue is directly incised and removed. In addition, a drug treatment method is used to remove cancer tissues that have not been completely removed after dissecting the cancer tissues, or to prevent cancer metastasis by remaining cancer cells and cancer tissues. Doxorubicin (Dox) which is used as an anticancer agent has excellent anticancer effects, but it is very toxic and has a short half-life in the human body, so the concentration and release of the drug must be well controlled. We have prepared a click-cross linkable hyaluronic acid hydrogel that can be directly injected into cancer to improve the efficacy of anticancer drugs and reduce toxicity by drugs. We individually introduced trans cyclooctene and tetrazine into hyaluronic acid (HA) so that the trans cyclooctene modified HA and tetrazine modified HA immediately formed Dox-containing hydrogel after being injected inside the tumor and the drug could be sustainably released. Cross-linked HA hydrogel consistently inhibited tumor growth, whereas non-crosslinked HA loaded with Dox and Dox solution increased the amount of Dox in the tumor and showed low anticancer activity. In conclusion, we successfully prepared a hydrogel capable of forming a drug depot within tumor and the anticancer effect of Dox loaded hydrogel could be increased.

Keywords

Hyaluronic acid; Click reaction; Drug release

Abstract **#954**

ENZYMATICALLY CROSSLINKED SILK FIBROIN HYDROGEL MICROFLUIDIC PLATFORM

Mariana Carvalho$_P$[1,2], Cristiana Carvalho[1,2], Raquel Maia[1,2], David Caballero[1,2], Subhas Kundu[1,2], Rui Reis[1,2], Joaquim Oliveira[1,2]

[1] *3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho*

[2] *ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal*

Corresponding author's email: mariana.carvalho@i3bs.uminho.pt

Microfluidic fabrication typically involves non-degradable materials including silicon and PDMS. However, these devices are generally not implantable, mainly because they are non-degradable and can induce inflammation and trigger foreign body reaction. Strategies for developing more natural implantable systems comprise the use of agarose, gelatin, collagen and alginate [1]. We have developed and patented a transparent enzymatically crosslinked silk fibroin hydrogel-based microfluidic device based on microfabrication techniques. It may be used ex vivo and in vivo, in tissue engineering applications, organ or tissue disease models, tissue-, organ- and body-on-a-chip, drug discovery, drug screening, tissue implant, tissue regeneration, and implantable microdevices. The new formulation is based on rapidly responsive silk fibroin hydrogels formed by a horseradish peroxidase (HRP) crosslinking reaction at physiological conditions, with potential use as an artificial biomimetic 3D ECM [2].

Preliminary results show the obtained hydrogel presents flexibility and elasticity, being organically adapted to any organ or structure in vivo. It is fully transparent until day 7, when its amorphous structure changes to β-sheet. The microfluidic serpentine feature is faithfully replicated into the hydrogel, and allows for perfusion of media, envisioning the formation of diffusion gradients of drugs/nanoparticles.

We envision mimicking colorectal cancer microenvironment by seeding human colonic microvascular endothelial cells inside the microchannels, invading the matrix in response to VEGF gradients, while HCT-116 colorectal cells are encapsulated in the silk. Due to its implantability, the system can be further implanted in more complex systems.

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Abstract **#956**

DEVELOPMENT AND CHARACTERIZATION OF A MICROSENSOR DEVICE FOR REAL TIME OXYGEN MONITORING IN 3D TISSUE ENGINEERED CONSTRUCTS

Daniele Zuncheddu[P1], Andrea Schwab[1], Elena Della Bella[1], Silvia Generelli[2], Felix Kurth[2], Pier Andrea Serra[3], Gaia Giovanna Maria Rocchitta[3], Hermann Kasper[1], Sibylle Grad[1], Valentina Basoli[1]

[1] AO Research Institute, AO Foundation, 7270 Davos, Switzerland

[2] Centre Suisse d'Electronique et de Microtechnique, Bahnhofstrasse 1, 7302 Landquart, Switzerland

[3] Department of Medical, Surgical and Experimental Sciences, University of Sassari, Viale San Pietro 43/b, 07100 Sassari, Italy

Corresponding author's email: Valentina.Basoli@aofoundation.org

The importance of oxygen concentrations in cell culture for tissue engineering and regenerative medicine is often underestimated1, although this parameter is crucial for cell fate during differentiation. The environmental oxygen concentration (20.9%) does not represent the physiological condition within human tissue2, such as bone and cartilage. For this reason, real time oxygen monitoring can improve the standardization of stem cell differentiation outcome, both for two-dimensional (2D) and three-dimensional (3D) methods. Therefore, we developed electrochemical oxygen sensors, already successfully applied in other fields3,4, using a copper wire inserted into a silica capillary tube, partly filled with a graphite-loaded epoxy resin. The sensors were introduced into the tissue plates through modified tissue plate lids, and the detection of oxygen was obtained by means of an electrochemical reduction, i.e. applying a cathodic potential to the carbon-disk surface. We tested the sensor stability in cell culture medium and its material cytotoxicity for bone marrow stem cells (BMSCs) cultures. The sensors are proved to be non-cytotoxic for up to 7 days. and were not damaged over a period of 21 days in cell culture medium The sensors were subsequently used to analyze oxygen concentration in 3D cultures of BMSCs embedded in two different hydrogels, gelatin methacryloyl (GelMa) and tyramine functionalized hyaluronic acid (HAT). The embedded stem cells were differentiated in chondrogenic medium for 21 days, both under hypoxic and normal conditions. The proposed system has the potential to represent an easy way of in real time oxygen concentration measurements in 3D constructs during differentiation.

Keywords

Oxygen microsensors; Real-time monitoring; 3D constructs

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Abstract **#957**

A 3D SPHEROIDS-BASED APPROACH TO MANUFACTURE AN IN VITRO ENGINEERED ARTICULAR CARTILAGE MODEL

Annachiara Scalzone[P1], Ana Marina Ferreira-Duarte[1], Xiao Nong Wang[1], Kenny Dalgarno[1], Piergiorgio Gentile[1]

[1] *Newcastle University*

Corresponding author's email: A.Scalzone2@newcastle.ac.uk

Articular cartilage (AC) repair is the main goal of several regenerative medicine approaches, with autologous chondrocyte implantation (ACI) providing an effective treatment[1]. A new variation on ACI called Chondrosphere™ (or Spherox; developed by CO.DON AG), based on the transplantation of autologous chondrocytes spheroids, has recently been approved and recommended by UK National Institute for Health and Care Excellence to treat symptomatic AC defects of up to 10 cm2[2]. Despite the current progress of Chondrosphere translation into clinical practice, the way the cells form spheroids, their development and their fusion when put together to mature into a tissue are still poorly understood. In this work we developed an in vitro AC repair model as a quality assessment tool for potency, which can be applied to Chondrosphere™ product[3,4]. To achieve the aim, we firstly analysed morphologically the way the cells aggregate in spheroids (by SEM and TEM), and biologically for testing their viability (Live/dead and Apoptosis/Necrosis assay) and metabolic activity (Cell Titer) during 7 days in vitro culture. Then we investigated the spheroids behaviours when placed together (10 spheroids per cm2 per each Chondrosphere) on a gelatin-coated Poly(ε-caprolactone) (PCL) membrane for 21 days. Spheroids tended to arrange in 3D cell aggregates, fusing among themselves and adhering to the gelatin-coated PCL membrane. Finally, chondrospheres showed to produce high amount of collagen II and aggrecan (qPCR) and GAGs (Alcian blue quantification) after long term in vitro cultivation, generating an AC-like tissue.

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Abstract **#958**

PHOTOCURABLE GELLAN GUM-BASED BIOINK ENRICHED WITH MANUKA HONEY FOR 3D-BIOPRINTED ENGINEERED ARTICULAR CARTILAGE CONSTRUCTS

Annachiara Scalzone[P1], Giorgia Cerqueni[2], Monica Mattioli-Belmonte[2], Xiao Nong Wang[1], Kenny Dalgarno[1], Ana Marina Ferreira-Duarte[1], Piergiorgio Gentile[1]

[1] *Newcastle University*

[2] *Università Politecnica delle Marche, Ancona, Italy*

Corresponding author's email: A.Scalzone2@newcastle.ac.uk

Three-dimensional bioprinting technology is predicted to radically change regenerative medicine, allowing the manufacture of tissue-engineered constructs with appropriate control over spatial variations and capability of precise deposition of biomaterials, cells and biological molecules[1]. One of the main challenges is to identify bioinks which can to fulfill the requirements for reproducible additive manufacturing as well as the biological requirements for the type of tissue required[2]. With regards to articular cartilage (AC) regeneration, hydrogel-based bioinks are the main formulations used due to their similarity to chondral tissue in terms of mechanical properties. In our work, we propose an innovative photocurable gellan gum methacrylated (GGMA) based bioink suitable for extrusion-based bioprinting technology to generate bioprinted AC tissues constructs of clinically relevant sizes. In particular, we exploited a nature-inspired strategy, adding Manuka Honey (MH) to the GGMA composition (GGMA-MH)[3]. The methacrylation efficiency of GGMA was chemically assessed by means of H-NMR and FTIR. Then, GGMA-based hydrogel formulations were used as chondrocyte laden bioinks, extruded to obtain a grid-shaped construct using a ROKIT INVIVO Bioprinter. The porosity of both compositions (GGMA and GGMA-MH) was investigated using SEM, together with water uptake analysis and mechanical properties via a compression test. The beneficial effect of Manuka Honey incorporation on printability and cells viability and fate was evaluated through cell viability assays. Histological analysis (H&E and Alcian blue stainings) and qPCR analysis (SOX9, COL2A1 and ACAN) were used to assess the chondrogenic potential of the cells-laden GGMA-based bioinks in vitro.

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Abstract **#959**

TISSUE ENGINEERED SPINAL CORD USING BONE MARROW STROMAL CELLS SEEDED POLYMERIC SCAFFOLDS

ILWOO LEE$_P$[1]

[1] *Depatment of Neurosurgery, Catholic University of Korea*

Corresponding author's email: leeilwoo@catholic.ac.kr

We tested the hypothesis that transplantation of bone marrow stromal stem cells (MSCs) into the spinal cord after a severe cord injury promotes functional outcome.

MSCs cells were harvested from adult Fischer rats and cultivated for transplantation. Before transplantation, MSCs were labeled with green fluorescent protein (GFP). 24 Fischer rats were anesthetized and laminectomy was performed at the T8~T9 level. A full thickness transection of the spinal cord was made at 2 levels, and 5 mm length transected segment of the spinal cord was removed. Cultured MSCs seeded with polyglycolide (PGA) scaffolds were transplanted into the gap of the spinal cord in 8 animals (group 1). Only PGA scaffolds without cells were transplanted into the gap of the spinal cord in 8 animals (group 2). No treatment was done in 8 animals (group 3). Motor functional outcome measurements using Basso-Beattie-Bresnehan (BBB) score were performed weekly to 8 weeks post-injury. Sections of spinal cord tissue including transplanted area were analyzed by H&E staining and immunohistochemical staining for NF, S-100 and NSE.

The results showed significant improvement in functional outcome in animals treated with MSCs transplantation (group 1) compared to group 2 or group 3. In histological analysis, evidence of axonal regeneration into the newly formed tissue at the gap was found in animals treated with MSCs transplantation (group 1), but very weak or no evidence of axonal regeneration was found in group 2 and group 3 animals. These data suggest that transplantation of MSCs may have a therapeutic role after SCI.

Keywords

bone marrow stromal cells; polyglycolide(PGA) scaffold; spina cord regeneration

Abstract **#962**

GLUTHATHIONE REDUCTASE- AND REDOX-RESPONSIVE POLYMERIC MICELLES FOR A TARGETED AND CONTROLLED DRUG DELIVERY IN ARTHRITIC DISEASES

Ana Cláudia Lima$_P$[1], Rui L. Reis[1], Helena Ferreira[1], Nuno M. Neves[1]
[1] *3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics*
Corresponding author's email: claudia.lima@i3bs.uminho.pt

The inadequate pharmacokinetic profile of arthritic diseases treatments, with low drug bioavailability and off-target biodistribution is a major limitation of their therapeutic efficacy and safety.1,2 Considering the tremendous health and economic impact of those conditions as well as the inflammatory microenvironment at the disease site, glutathione reductase (GR)- and redox-responsive polymeric micelles were designed to provide a targeted and controlled drug release. To establish a sensitive system, a novel polymer composed of methoxypolyethylene glycol amine-glutathione-palmitic acid (mPEG-GSH(n)-PA) was synthetized and after the micelles formation the thiol groups were oxidized intermolecularly to avoid drug leakage under physiological conditions. After passive targeted accumulation in inflamed joints, redox environment and GR activity will destabilize the micellar structure via a thiol-disulfide exchange, triggering a local and controlled drug release.
The micelles presented a uniform size of around 110 nm and 65% entrapment efficiency of dexamethasone (Dex). After demonstrating their hemocompatibility and cytocompatibility in contact with human endothelial cells, chondrocytes and macrophages, in a co-culture model of inflammation and in the presence of GR, polymeric micelles promoted a targeted and controlled drug release. Importantly, as they presented a higher efficacy than the free Dex and were able to reduce its negative effects over normal cells, this strategy may provide important outcomes in arthritis treatment. In conclusion, by increasing the drug therapeutic efficacy and reducing its severe side effects the developed polymeric micelles offer unique advantages for the treatment of arthritic diseases.
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Keywords
Enzymatic- and Redox-responsive Micelles; Targeted and Controlled Drug Delivery; Arthritic diseases

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Abstract **#963**

CHARACTERIZING THE EFFECTS OF PHYSIOLOGICAL OXYGEN CONCENTRATIONS ON KIDNEY ORGANOID MATURATION AND ENDOTHELIALISATION

Anika Schumacher[P][1], Nadia Roumans[1], Timo Rademakers[1], Martijn van Griensven[1], Vanessa LaPointe[1]

[1] *Department of Cell Biology–Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands*

Corresponding author's email: anika.schumacher@maastrichtuniversity.nl

Organoids are gaining significant interest in the field of regenerative medicine. Their ability to self-organize from pluripotent stem cells into functional organ-like structures makes them candidates for organ replacement or repair. We aim to produce functional kidney organoids from induced pluripotent stem cells (iPSCs) to build an implantable kidney graft to reduce or replace dialysis for patients with end-stage kidney disease. For this, we differentiate iPSCs and subsequently form organoids by aggregation. After 18 days of culture, these are several millimeters in size and comprise small nephron-like structures, with tubular segments and an immature endothelium. We found that prolonged culture results in diminishing endothelial cells and deteriorating nephrons. Notably, the organoids grow in a transwell setup at an air-liquid interface, and are therefore directly exposed to a hyperoxic (21%) culture environment. This culture method has been used to culture kidney explants since the 1950s, however, there is little understanding of its effects. To gather more insight, we cultured our organoids in a hypoxic environment similar to the physiological range of oxygen in developing human kidneys. We investigate its effect on HIF1/2 pathway activation and hypothesize these pathways lead to an enhanced transcription of VEGFa, consequently enriching the endothelial cell population in the organoid. To confirm transcription of HIF target genes, we use techniques such as qPCR and whole mount imaging facilitated by tissue clearing. Ultimately, we aim to understand the effect of culturing kidney organoids in physiological oxygen concentrations.

Abstract **#964**

NOVEL STRATEGIES TO PROMOTE HUMAN EMBRYONIC STEM CELL CHONDROGENESIS IN 3D MICROENVIRONMENTS

Miguel J.S. Ferreira[P][1], Christopher A. Smith[2], Samuel R. Moxon[3], Michael Buckley[4], Susan J. Kimber[2], Marco A.N. Domingos[1,5]

[1] Department of Mechanical, Aerospace and Civil Engineering, School of Engineering, Faculty of Science and Engineering, The University of Manchester, UK

[2] Division of Cell Matrix Biology and Regenerative Medicine, Faculty of Biology, Medicine and Health, The University of Manchester

[3] Division of Neuroscience and Experimental Psychology, Faculty of Biology, Medicine and Health, The University of Manchester, UK

[4] Manchester Institute of Biotechnology, The University of Manchester, UK

[5] The Henry Royce Institute, Alan Turing Building, Oxford Road, Manchester, M13 9PL, United Kingdom

Corresponding author's email: miguel.ferreira@postgrad.manchester.ac.uk

Recently, protocols to differentiate human embryonic stem cells (hESCs) into the chondrogenic lineage have been established [1,2]. However, generating 3D articular cartilage tissue from hESCs is still challenging.

This work aims to develop a bioprinted articular cartilage model using hESCs, through the combination of alginate-based hydrogels as an ECM-like microenvironment for cell differentiation, and L-Leucine-based Poly(ester urea) (PEU) scaffolds with adequate biophysical properties to withstand physiological loading.

First, hESCs were differentiated into the chondrogenic lineage using a 2D multi-step protocol. Then, hESC-derived chondroprogenitors were encapsulated in alginate or alginate/type-I collagen hydrogels. Differentiation into the chondrogenic lineage was verified by changes in cell morphology, loss of expression of pluripotency markers, and increased expression of chondrogenic markers SOX9, SOX5, and COL2A1 during 2D differentiation. Further culture in 3D hydrogels for 14 days resulted in high levels of cell viability and increased chondrogenic gene expression.

Then, the ability of bioprinted PEU scaffolds to support chondrogenesis was evaluated. PEU scaffolds had lower nanoindentation hardness and compressive modulus, and were more hydrophilic than poly(caprolactone) (PCL) controls. Human chondrocytes cultured on PEU exhibited increased metabolic activity, expression of chondrogenic markers ACAN and COL2A1, and lower expression of COL1A1, suggesting a more chondrogenic phenotype.

These findings suggest that alginate-based hydrogels are suitable systems to promote chondrogenesis from hESCs. Similarly, PEUs were able to support human chondrocyte phenotype in culture. The combination of these two components could be applied to generate a 3D bioprinted hybrid construct for new applications in cartilage regenerative therapies or in vitro tissue modelling.

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Abstract **#965**

MULTIPLE AND SIMULTANEOUS MEASUREMENTS OF 3D CELL CULTURE VIABILITY USING MINIATURE ELECTRICAL IMPEDANCE TOMOGRAPHY SENSOR

Ryoma Ogawa[P][1,2], Pierre Bagnaninchi[1], Jiabin Jia[3], Hancong Wu[4], Amelia Hallas-Potts[5]

[1] *Medical Research Council Centre for Regenerative Medicine, The University of Edinburgh, UK*

[2] *Graduate School of Science and Engineering, Chiba University, Japan*

[3] *School of Engineering, The University of Edinburgh*

[4] *School of Engineering, Newcastle University*

[5] *Edinburgh Cancer Research Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh*

Corresponding author's email: afna2097@chiba-u.jp

There is a need for noninvasive monitoring of cell culture growing and differentiating in bioscaffolds to evaluate their viability and quality before their eventual transplantation to patients.
However, current cell viability assays are mostly limited to 2D cell culture and make use of destructive imaging technologies (e.g. histology) or chemical probes that are not ideally suited for clinical translation. Here, we developed a biocompatible scaffold that mimics the extracellular matrix and holds the 3D cell culture medium with a design that both optimizes the surface tension power, and allow the assessment of the cell culture with Electrical impedance tomography. We characterized our scaffold as a frequency-independent dielectric material, and we demonstrated both in silico and in vitro that frequency-dependent electrical impedance tomography (fd-EIT) can image and measure in parallel several scaffolds in a single EIT miniature sensor. We retrieved the cell concentration of up to four different scaffolds non-destructively in real-time. Furthermore, quantitative analysis in the field of current density and potential distribution was conducted to explain the feasibility of cell differentiation using fd-EIT. This study paves the way towards EIT imaging and measurements that could help to evaluate quantitatively and noninvasively tissue engineering samples in the production line.

Keywords

Electrical Impedance Tomography; cellular assay; biocompatible scaffolds

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Abstract **#966**

MODULATION OF CARDIAC FIBROBLAST ACTIVATION THROUGH LACTATE-BASED BIOMATERIALS FOR IN SITU CARDIAC REGENERATION

Marina Martínez-Hernández[P][1,2,3], Jesús Ordoño[1,4], Laura Tuduri[1], Oscar Castaño[5,1,4], Soledad Pérez-Amodio[1,3,4], Elena Martínez[2,4,5], Elisabeth Engel[3,1,4]

[1] *Biomaterials for regenerative therapies, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain*

[2] *Biomimetic systems for cell engineering, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain*

[3] *Materials Science and Metallurgical Engineering, Polytechnical University of Catalonia (UPC), Barcelona, Spain*

[4] *Centro de Investigación Biomédica en Red en bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain*

[5] *Department of Electronics and Biomedical Engineering, University of Barcelona (UB), Barcelona, Spain*

Corresponding author's email: mmartinez@ibecbarcelona.eu

INTRODUCTION: Lactate, an important metabolite during cardiogenesis and cardiac development, has been recently shown to promote cardiomyocyte proliferation and reprogramming towards a dedifferentiated stem cell-like state.1 Non-myocytic cardiac fibroblasts (CFBs) have a key role during cardiac remodeling after myocardial infarction, where they become activated and release extracellular matrix proteins. Excessive extracellular matrix deposition or myofibroblast activation is detrimental to cardiac repair.
The aim of this work is to elucidate the effect of lactate on CFBs for a complete interpretation of its potential proregenerative capabilities. Then, we developed bioactive lactate-releasing scaffolds for cardiac regeneration.

METHODS: CFBs metabolic activity and proliferation were assessed. The inflammatory and fibrotic responses to exogenous lactate were investigated in terms of cytokine and collagen production, migration assays and myofibroblast differentiation. Then, polylactic-acid(PLA) scaffolds were fabricated by electrospinning and physiochemically characterized (SEM, DSC, lactate release,…).

RESULTS: Our results indicate that lactate does not affect fibroblast proliferation, migration, collagen production, or activation and significantly reduces the expression of detrimental cytokines for cardiac repair. Furthermore, our PLA scaffolds are amorphous, biodegradable, nanofibrous materials that release lactate in a sustained manner. They do not affect CFB viability and promote cell attachment.

DISCUSSION: Altogether, this study further supports the prospective use of lactate as a bioactive signal in new endogenous cardiac regeneration therapies. Thus, PLA patches that release lactate as a major product from its breakdown can then be used as an effective source of lactate for alleviating the aftermath of myocardial infarction.

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Abstract **#967**

HOW BUILDING ON ESTABLISHED TECHNOLOGY CAN FAIL: THE PROBLEMS WITH AN OSTEOCHONDRAL IMPLANT BASED ON A PROVEN BONE COMPONENT EVALUATED IN VIVO

Florencia Abinzano[P][1], Paweena Diloksumpan[2], Mylène de Ruijter[1], Anneloes Mensinga[1,2], Saskia Plomp[2], Ilyas Khan[3], Harold Brommer[2], Ineke Smit[2], Miguel Dias Castilho[1], René van Weeren[2], Jos Malda[1,2], Riccardo Levato[1,2]

[1] *Department of Orthopaedics, University Medical Center Utrecht, Utrecht University*

[2] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University*

[3] *Center for Nanohealth, Institute of Life Science, College of Medicine, Swansea University*

Corresponding author's email: f.abinzano@umcutrecht.nl

Repair of articular cartilage defects remain a major clinical challenge, since there are currently no long-term regenerative options. Implant-based approaches have shown promise but the fixation of the constructs is problematic.[1]The aim of this study was to evaluate the performance of an integrated osteochondral implant in an orthotopic equine model. The construct consisted of a 3D printed low-temperature setting bioceramic bone anchor which was firmly integrated to a cartilage-mimetic compartment consisting of a melt-electrowritten polycaprolactone mesh,[2] with or without seeded articular cartilage progenitor cells. The bone anchor had been previously shown to induce osteoregeneration when implanted in vivo into the tuber coxae of warmblood horses.[3] To maximize cartilage matrix production, the constructs were precultured for 28 days in media supplemented with bone morphogenetic protein-9, resulting in abundant extracellular matrix rich in glycosaminoglycans and type II collagen. The osteochondral plugs were then implanted in the stifle joints of Shetland ponies. Radiographic examinations and quantitative gait analysis were implemented to monitor clinical status. Six months after implantation, histological and biochemical analyses showed minimal amounts of GAGs and type II collagen in the chondral region for cell-laden and cell-free implants, while the microfiber meshes were still present. Quantitative micro-computed tomography showed collapse of the bone anchor and low volume of mineralized neo-bone formation in both groups. It was concluded that the failure of the bone component resulted in the loss of mechanical support and improper fixation, which affected the overall outcome, hindering the evaluation of BMP-9 stimulated cells for in vivo cartilage repair.

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Abstract **#968**

3D BIOPRINTING OF QUALITY CONTROL STANDARDS FOR MASS SPECTROMETRY

Berta Cillero-Pastor[P][1], Erik van Zetten[1], Monica Basílio de Sousa Carvalho[2], Carlos Domingues Mota[2]

[1] Maastricht MultiModal Molecular Imaging (M4I) institute, Division of Imaging Mass Spectrometry, Maastricht University, NL

[2] MERLN Institute for Technology-inspired Regenerative Medicine, Complex Tissue Regeneration Department, Maastricht University, Maastricht, The Netherlands

Corresponding author's email: b.cilleropastor@maastrichtuniversity.nl

Mass spectrometry imaging (MSI) has become a powerful method for unbiased tissue-based disease and patient classification (1). By scanning a tissue biopsy with a laser, individual mass spectra are recorded from predefined coordinates providing, within a short time, a detailed spatial molecular fingerprint (2). Recent instrumental improvements have resulted in scan times of just several minutes, making it an ideal candidate for new digital pathology workflows while allowing a fast-clinical diagnosis and intervention in an (unprecedented) precise manner (3). A key factor in the implementation of MSI for routine Digital Molecular Pathology Diagnosis, is the need of robust and precise quality control standards (QCS). Most laboratories employ liquid based (commercially available) compounds, animal-derived organ sections or tissue homogenates. All these solutions are not adequate as QCS, because of batch-to-batch differences, organ/animal heterogeneity and insufficient resemblance with human tissue molecular complexity. The ideal QCS for MSI technology should mimic the structure of biological tissues: a cellular component embedded in an extracellular matrix (ECM). Within our team, we have developed an elegant solution that meets the sketched requirements by 3D bioprinting engineered tissue constructs capable of mimicking both the molecular and topographical biology characteristics for MSI applications.

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Abstract **#969**

GUIDED CELL MIGRATION AND VASCULAR SPROUTS FORMATION THROUGH HIGH-DEFINITION LASER GRAFTING

Tommaso Zandrini$_P$[1,2], Simon Sayer[1,2], Marica Markovic[1,2], Jasper Van Hoorick[3], Orestis Andriotis[4], Philipp Thurner[4], Sandra Van Vlierberghe[3], Aleksandr Ovsianikov[1,2]

[1] *Institute of Materials Science and Technology, TU Wien*

[2] *Austrian Cluster for Tissue Regeneration*

[3] *Polymer Chemistry and Biomaterials Group, Department of Organic and Macromolecular Chemistry, Ghent University*

[4] *Institute of Lightweight Design and Structural Biomechanics, TU Wien*

Corresponding author's email: tommaso.zandrini@tuwien.ac.at

The formation of vascular sprouts from endothelial cells (ECs) is a necessary step for the creation of micro-vascular networks[1]. To proliferate and sprout, ECs need support from stem cells, degrading the extracellular matrix and providing cues[1–3]. Spontaneous formation of sprouts in hydrogels has already been shown[1], but a fine spatial control of their direction remains challenging.

We used a co-culture of spheroids from human umbilical vein ECs (HUVEC) and adipose stem cells (ASC), embedded in a methacrylamide-functionalized gelatin (GelMA)[4], cross-linked with UV light, as a platform for ECs sprouts formation. After polymerization, the gel has been soaked in 4,4'-diazido-2,2'-stilbenedisulfonic acid (DSSA), and channel-shaped structures have been patterned around the spheroids with an infrared femtosecond laser. DSSA is grafted to the hydrogel backbone only in the patterned regions, locally modifying stiffness and hydrophobicity.

In the following week, we observed preferential migration and alignment of ASCs along the grafted channels, with faster migration and higher cell density compared to non-grafted regions. Also, migration speed increased at higher laser power. HUVECs sprouts formed in the regions with a high number of ASCs, both the grafted channels, and the immediate surroundings of the spheroids, where ASCs spontaneously migrated.

We can conclude that the localized modulation of the mechanical and physical properties of gelatin-derived materials through femtosecond laser light is a powerful tool to guide the growth and spatial organization of support stem cells and endothelial cells. The synthesis and selection of appropriate biocompatible materials will be critical for the advancement of this technique.

Keywords

High Definition Bioprinting; Vascular sprouts; Femtosecond laser

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Abstract **#970**

IN SITU ENGINEERING OF A NOVEL HYBRID SILK FIBROIN/POLYURETHANE ARTERIOVENOUS GRAFT FOR HEMODIALYSIS: PROOF-OF-CONCEPT ANIMAL STUDY IN AN OVINE MODEL

Stefania Adele Riboldi[P][1], Matteo Tozzi[2], Mara Bagardi[3], Giuliano Ravasio[3], Giorgio Cigalino[3], Luca Crippa[4], Solange Piccolo[2], Amal Nahal[2], Mattia Spandri[1], Valentina Catto[1], Matteo Tironi[5], Francesco Giovanni Greco[1], Andrea Remuzzi[6], Fabio Acocella[3]

[1] *Bioengineering Laboratories S.r.l., Cantù, Italy*

[2] *Università degli Studi dell'Insubria, Varese, Italy*

[3] *Università degli Studi di Milano, Milano, Italy*

[4] *Università degli Studi di Milano Bicocca, Milano, Italy*

[5] *IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy*

[6] *Università degli Studi di Bergamo, Bergamo, Italy*

Corresponding author's email: sriboldi@dialybrid.com

To solve the problem of vascular access failure, a novel semi-degradable hybrid vascular graft, manufactured by electrospinning using silk fibroin and polyurethane (Silkothane®), was previously developed and characterized in vitro. In this proof-of-concept animal study, we aimed at evaluating the performances of Silkothane® grafts in a sheep model of arteriovenous shunt, in terms of patency and short-term graft remodeling. Nine Silkothane® grafts were implanted between the common carotid artery and the external jugular vein of nine sheep, which were examined by palpation three times per week, by echo-color Doppler every two weeks, and euthanized at 30, 60 and 90 days (N=3 per group). At sacrifice, graft and anastomoses were harvested and submitted for histopathology and/or scanning electron microscopy (SEM). No cases of graft-related complications were recorded in this study. Eight of nine sheep (89%, one case of surgery-related thrombosis excluded) showed 100% primary unassisted patency at the respective time of sacrifice (flow rate 1.76 ± 0.61 L min-1). Histopathology and SEM analysis evidenced signs of inflammation and pseudointima inside the graft lumen, especially at the venous anastomosis, as it typically happens with other grafts; however, endoluminal stenosis never impaired the functionality of the shunt and coverage by endothelial cells was observed. In our model, Silkothane® grafts granted safety and 100% patency up to 90 days, paving the way for possible in situ engineering strategies of vascular accesses for hemodialysis, aiming to enable early cannulation as synthetic grafts and superior long-term performances as native fistulae.

Keywords

Silk fibroin; Vascular graft; Hemodialysis

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Abstract **#971**

IMMUNO-MODULATORY AND PRO-ANGIOGENIC EXTRACELLULAR MATRIX COMBINATIONS FOR LARGE DEFECT PERIPHERAL NERVE REPAIR IN RAT AND RABBIT MODELS

Alan Hibbitts[P][1,2,3], Zuzana Koci[1,2,3], Simone Kneafsey[1,2,3], Leyla Zilic[1,2,3], Adrian Dervan[1,2,3], Paige Hinton[1,2,3], Gang Chen[4], Brenton Cavanagh[5], Conor Buckley[1,2,3], Simon Archibald[6], Fergal O'Brien[1,2,3]

[1] Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland.

[2] Trinity Centre for Biomedical Engineering, Trinity College Dublin, Dublin, Ireland.

[3] Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland.

[4] Department of Physiology and Medical Physics, Centre for the Study of Neurological Disorders, Microsurgical Research and Training Facility (MRTF), RCSI, Dublin, Ireland

[5] Cellular and Molecular Imaging Core, RCSI, Dublin, Ireland.

[6] Integra LifeSciences, Plainsboro, NJ, USA

Corresponding author's email: alanhibbitts@rcsi.ie

Large defect repair using nerve guidance conduits (NGCs) remains inconsistent (1). To address this, we have developed a filled NGC with an internal matrix consisting of microporous channels infused with macromolecules abundant in nerve extra-cellular matrix (ECM).

NGCs were produced using an outer collagen conduit and an internal matrix of collagen-chondroitin-6-sulphate (Coll-CS) augmented with ECM combinations of fibronectin, laminin I and laminin II. ECM combinations were assessed for growth and inflammatory biomarkers using primary rat dorsal root ganglia (DRG) cultures over 14 days. Following this, large 15 mm sciatic nerve defects were created in rats and lead candidate NGCs were implanted for 8 weeks. This was again assessed at the clinical limits of efficacy in a 30 mm sciatic nerve defect in rabbits at 6 and 12 weeks. Regeneration was assessed using electrophysiology and immuno-histochemistry.

Highly porous and unidirectional NGCs were formed which significantly improved expression of repair and inflammatory cytokines using ex-vivo DRG culture. When lead candidates were progressed to rat models, regenerating tissue was found to be present throughout the NGC and functional recovery was comparable to autograft controls. Similarly, at 6 weeks post-implantation, numerous myelinated axons were found to be present at over 15 mm distance from the proximal nerve in rabbit models.

The pro-regenerative effects of ECM in peripheral nerve repair were highly combination-dependent. Most promisingly, surgical outcomes were found to be comparable to autograft controls using optimised ECM-NGCs

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Abstract **#972**

BIOMECHANICAL EVALUATION OF A TISSUE ENGINEERED INTERVERTEBRAL DISC REPAIR PATCH IN AN OVINE MODEL

Mitchell Page[P][1], Christian Puttlitz[2]

[1] *Orthopaedic Bioengineering Research Laboratory and Translational Medicine Institute, Department of Mechanical Engineering, Colorado State University*

[2] *Orthopaedic Bioengineering Research Laboratory and Translational Medicine Institute, Department of Mechanical Engineering, Colorado State University*

Corresponding author's email: mitchell.page@colostate.edu

Regenerative strategies for repair of the intervertebral disc's (IVD) annulus fibrosus (AF) require the delivery of mechanical stimuli to resident cells as well as reconsolidation of IVD biomechanics. Accordingly, our group has developed an AF repair scaffold using a previously developed biomimetic architecture and fabricated the implants via fused deposition modelling (FDM) of polycaprolactone (approximately 300µm diameter fibers). Functional spine units (FSUs; level four and five) were dissected from eight (n=8) sheep for biomechanical testing in ±6Nm pure moment loading. Each FSU was tested in three conditions: (1) intact, (2) with an 8mm AF defect, and (3) treated with the repair patch. To assess the regenerative potential of the implant, a computational model was used to predict deformation of the implant and subsequent mechanobiological response. The defect group generated significant increases in FSU range of motion and significant decreases in FSU neutral zone stiffness (α=0.05 for all comparisons). In all cases, the treated group recovered some of the deleterious changes induced by the defect. The predicted implant deformations indicated compressive radial and axial strains (-3.2% to -7.5% and -0.4% to -16.9%, respectively) and tensile circumferential strains (0.2% to 5.0%). These results suggest that a FDM implant can restore spinal biomechanics following an annular defect. However, the associated cellular scale mechanics within the implant may not be conducive for regeneration [2]. Overall, in order to tailor the mechanical loading of AF implants, this work suggests that advanced implant designs and novel attachment techniques (e.g., bioadhesives) may be necessary for effective regeneration.

Keywords

annulus fibrosus; repair patch; spine biomechanics

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Abstract **#973**

3D IN VITRO T-CELL ATTACHMENT AND DYNAMIC BIOREACTOR CULTURE FOR EXPLORING EXOSOME INDUCED IMMUNOSUPPRESSION

Vassilios Sikavitsas[P][1], Daniel Karami[1]

[1] *University of Oklahoma*

Corresponding author's email: vis@ou.edu

Immunotherapy has emerged as a novel approach to treat cancer utilizing the patient's own immune system that manages to attack effectively cancer cells. Tumors that are naturally infiltrated by white blood cells like brain tumors, melanomas, kidney cancer, lung cancer, and cervical cancer among others have been the obvious targets of immunotherapy approaches. Immunotherapy approaches include treatments that target the immune system checkpoints. The success of immunotherapy treatments have been linked to the successful activation of the patient's immune system and the tumor microenvironment. The tumor microenvironment includes exosomes released by cancer cells. Exosomes are small membrane vesicles originating from the cell cytoplasm that are released to their microenvironment, carrying parts of the cell membrane. T-cells are responsible for many of the cytotoxic responses in the body, but due to hypothesized immunosuppressive properties of the exosomes they can be silenced. We explored the interactions of cancer cell generated exosomes with activated T-cells in traditional 2D flasks and 3D cultures where T-cells were attached on RGD-modified scaffolds in the presence of flow perfusion. IL-2, a common proliferation cytokine, is released by PMA stimulated T-cells, and the response of T-cells can be correlated to their cytokine production. When co-cultured with exosomes from H1299 and A549 cancer cell lines, T-cells showed decreased IL-2 release when increasing the exosomes from a 1:10 ratio to 1:1000 ratio in 3D cultures with flow perfusion rates of 0.15 mL/min. Our system indicated exosome induced immunosuppression and provides an attractive environment to further explore T-cell immunosuppression in vitro.

Abstract **#974**

HISTOPATHOLOGICAL AND RADIOGRAPHICAL EVALUATION OF CAPRINE BONE XENOGRAFT IN A CRITICAL ULNAR DEFECT IN A RABBIT MODEL

Olawale Alimi Alimiᴘ[1,2], Adamu Abdul Abubakar[2], Abubakar Sadiq Yakubu[2], Sani Abdullahi Shehu[3], Salman Zubairu Abdulkadir[1,2]

[1] *Department of Veterinary Surgery and Radiology, University of Ilorin, Ilorin, Nigeria*

[2] *Department of Veterinary Surgery and Radiology, Usmanu Danfodiyo University, Sokoto, Nigeria*

[3] *Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto, Nigeria*

Corresponding author's email: alimialimi@gmail.com

Caprine species satisfy the conditions of an ideal donor animal when compared to bovine species that has been extensively studied and commercialized for bone xenograft. Histopathological and radiological evaluations of caprine demineralized bone matrix (CDBM) were therefore carried out for fracture healing properties for its possible use in bone grafting. Twenty-four rabbits were divided randomly into three groups of eight (n=8) rabbits each. Critical bone defect was created on the ulnar diaphysis under xylazine-ketamine anaesthesia for autogenous bone graft (ABG) group, CDBM group and the unfilled group as the negative control (NC). Immediate post-grafting radiograph was taken and repeated on days 14, 28, 42 and 56 to monitor the evidence of radiographic healing. The animals were euthanized on day 56 and defect sites harvested for histopathology. There was progressive evidence of radiographic healing and bone formation in all the groups with significance difference (P=0.0064). When compared with ABG, NC differ significantly (P<0.0001) whereas the CDBM did not differ significantly (P=0.6765). The histopathology sections of ABG and CDBM showed normal bone tissue while the NC section was predominated by fibrous connective tissue. There was, therefore, an overall significant difference (P=0.0001) in which CDBM did not differ from ABG (P=0.2946) while NC did (P=0.0005). The ABG and CDBM groups appeared to have the same healing effect in the critical bone defect. Therefore, CDBM could be used as an effective alternative to ABG in orthopaedics to circumvent the limitations and complications associated with it.

Keywords: Bone grafting, Bone xenograft, Demineralized bone matrix

Abstract **#975**

AICAR ENHANCES THE PROLIFERATION OF MSCS INITIALLY, BUT IN THE LONG RUN, IT IS ANTI-PROLIFERATIVE!

Mohammadhossein Khorraminejad-Shirazi[P][1,2], Mahsa Sani[3], Armin Attar[4], Mohammadreza Dorvash[2], Tahereh Talaei-Khozani[5]

[1] *Student research committee, Shiraz University of Medical Sciences, Shiraz, Iran*

[2] *Cell and Molecular Medicine Student Research Group, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[3] *Tissue Engineering Department, School of Advanced Medical Science and Technology, Shiraz University of Medical Science, Shiraz, Iran*

[4] *Department of Cardiovascular Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[5] *Tissue Engineering Lab, Department of Anatomical Sciences, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

Corresponding author's email: hkhorraminejad@gmail.com

Mesenchymal stem cells (MSCs) are limited by the number of passages in-vitro due to aging (1). In our recent study (2), we used 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) and nicotinamide (NAM) to attenuate the senescence of MSCs. We demonstrated that AICAR and NAM retain the proliferative capacity of cells lost after prolonged in-vitro culture. To further evaluate the effect of AICAR and NAM on the proliferation of MSCs, we performed MTT assay on passage-10 MSCs, ten days after cell seeding. Our results show that while cell proliferation was increased with NAM treatment ($p<.05$), AICAR significantly inhibited the growth of the cultured cells ($p<.05$) compared to the untreated cells.

To further discuss our findings, as we presented in our study (2), AICAR, NAM, and their combination dramatically increased the number of cells after seven days. However, as we further culture MSCs in the presence of AICAR, the cell density drops after ten days, indicative of the anti-proliferative effect of AICAR after extended in-vitro culture. This observation might be in light of the higher apoptosis rate in AICAR-treated cells. To elucidate, the percentage of Annexin-V positive cells was almost two times that of the untreated cells, and approximately three times higher than the NAM-treated cells. This most likely happens due to a molecular switch between autophagy and apoptosis; if autophagy cannot prevail the damages, then apoptotic mechanisms terminate that cell (3,4). In other words, while AICAR augments autophagy in MSCs to prevent senescence, it increases the rate of apoptosis, as well.

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Abstract **#976**

TEMPERATURE EFFECTS ON BIO-PRINTABILITY OF GELATIN-METHACRYLOYL BIOINK

MOHSEN JANMALEKI$_P$[1], Milad Kamkar[1], Milad Azarmanesh[1], Uttandaraman Sundararaj[1], Amir Sanati Nezhad[1], Jun Liu[1]

[1] *University of Calgary*

Corresponding author's email: mohsen.janmaleki@ucalgary.ca

In extrusion-based bioprinters, the stable formation of filaments and high-fidelity deposition of bioinks are the primary challenges in fabrication of physiologically relevant tissue constructs. The two-step crosslinking of Gelatin-Methacryloyl (GelMA), reversible thermal gelation and permanent photo-crosslinking, has attracted researchers to make complex tissue constructs. Despite promising results in filament formation and printability of this hydrogel, the effect of temperature on physicochemical properties, cytocompatibility, and biodegradation of the hydrogel needs to be investigated. The results of 3D printing of GelMA at different temperatures followed by an irreversible chemical photo-crosslinking show that the decrease in temperature improves the filament formation and shape fidelity of the deposited hydrogel, particularly at the temperatures around 15 °C. Time dependant mechanical testing of the printed samples revealed that decreasing the extruding temperature increases the elastic properties of the extruded filaments. Furthermore, our novel approach in minimizing the slippage effect during rheological study enabled us to measure changes in linear and nonlinear viscoelastic properties of the printed samples at different temperatures. A considerable increase in storage modulus of the extruded samples printed at lower temperatures confirms their higher solid behavior. Scanning electron microscopy revealed a remarkable decrease in porosity of the extruded hydrogels by decreasing the temperature. Chemical analysis by Fourier-transform infrared spectroscopy (FTIR) and circular dichroism (CD) showed a direct relationship between the coil-helix transition in hydrogel macromers and its physical alterations. Finally, biodegradation and cytocompatibility of the extruded hydrogels decreased at lower extruding temperatures.

Abstract **#977**

MAGNETIC FIELD-ASSISTED ELECTROSPINNING: A MATHEMATICAL STUDY

MOHSEN JANMALEKI$_P$[1], Saeide Badieyan[2]

[1] *University of Calgary*

[2] *Shahid Beheshti University of Medical Sciences*

Corresponding author's email: mohsen.janmaleki@ucalgary.ca

Electrospinning is known as an efficient and straightforward approach to fabricate of extremely thin fibers from a rich variety of polymers. Unpredictable deposition of generated nanofibers has limited its applications for precise and reproducible biomaterial fabrications. In this study, a magnetic field-assisted approach has been introduced and its practicality has been evaluated through mathematical modelling. The developed model was employed to study the behavior of the generated polymeric jet numerically using the Runge-Kutta method. The jet was assumed to consist of a number of discrete charged particles connected by viscoelastic segments. The results showed that exerting an appropriate magnetic field (MF) could significantly decrease the radius and the instability of the whipping circles. After fixing the instability as far as possible, it was demonstrated that a properly applied perpendicular MF could largely adjust the target of the polymer jet on the collector.

Abstract **#978**

PROGRESS AND FUTURE PROSPECTS OF CLINICAL RESEARCH ON SCAFFOLD-FREE BIOFABRICATION BY THE KENZAN METHOD

Koichi Nakayama[P][1]

[1] *Saga university*

Corresponding author's email: nakayama@me.saga-u.ac.jp

In this symposium, we will introduce our original biofabrication system called "Kenzan method" including the first clinical results of blood vessel regeneration done in Saga university hospital, and other pipelines which are getting closer to clinical application.

As far as we know, this would be the world first clinical application made with scaffold-free bio 3D printing system.

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Abstract **#980**

3D BIOPRINTING COMPLEX TISSUE MODELS FOR DRUG SCREENING

Stephanie Willerth[P][1]

[1] *University of Victoria*

Corresponding author's email: willerth@uvic.ca

3D bioprinting can create living human tissues on demand based on specifications contained in a digital file. Such highly customized, physiologically-relevant 3D human tissue models can screen potential drug candidates as an alternative to expensive pre-clinical animal testing. The Willerth lab has developed a novel fibrin-based bioink for bioprinting neural tissues derived from human induced pluripotent stem cells (hiPSCs), which can become any cell type found in the body. Our team uses Aspect Biosystem's novel RX1 bioprinter featuring Lab-On-a-Printer™ (LOP) technology as it enables us to fabricate complex structures found in healthy neural tissues. We were the first group to use this cutting edge bioprinter to generate tissues from pluripotent stem cells. The microfluidic LOP™ printhead cartridges generate cell-containing hydrogel fibers of defined diameters that are precisely deposited into defined 3D structures using a sheath fluid that triggers hydrogel cross-linking of the bioink. The sheath fluid also insulates cells within the fiber from shear stress, protecting fragile primary cells from shear-induced cell death. This process allows us to maintain high levels of viability (>90% post printing) not previously seen in the literature. Here I will discuss the latest work from our group detailing the composition of our 3D bioprinted tissues and our new spin-off company - Axolotl Biosciences.

Abstract **#981**

HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION INTO RETINAL PIGMENTED EPITHELIUM PROGENITORS ON ECM-COATED DISHES

Yu Ru Zhu[P][1], Ming Wei Lu[1], Akon Highchi[1,2]

[1] *Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli,Taoyuan 32001, Taiwan*

[2] *Nano Medical Engineering Laboratory, RIKEN, Wako, Saitama351-0198, Japan*

Corresponding author's email: lihibarike110391255@gmail.com

Introduction:

Age-related macular degeneration (AMD), according to world health organization, ranks the third leading cause of vision impairment and further causes irreversible blindness. This AMD disease mainly results from the dysfunction of retinal pigmented epithelium (RPE). Fortunately, transplanting human pluripotent stem cells (hPSCs)-derived RPE can serve as regenerative approach to cure AMD disease. However, RPE derived from hPSCs usually suffers from insufficient purity, long culture period and low yield. Therefore, we compared different protocols and cell culture extracellular matrices (ECMs) to investigate which conditions would be the most suitable for hPSCs to differentiate into RPE progenitors.

Method:

We first selected and compared the protocols, (i) N2 protocol [1] and (ii) NIC+CTM protocol [2]. After we found the better protocol, we tried different substrates to culture and induce differentiation of hPSCs into RPEs. We evaluated neural marker of PAX6 expression by using immunostaining and flow cytometry assay to find the best condition for hPSCs to differentiate in to RPE progenitors.

Results and Discussion:

According to epidermal marker of PAX6 analysis from immunostaining and flow cytometry assay, we found NIC+CTM protocol showed the better performance of hPSC differentiation into RPEs compared to N2 protocol. Subsequently, we found the optimal xeno-free culturing surface that could be applied for massive production of high purity of RPE progenitors in NIC+CTM protocol. We are planning to use the same protocol to further investigate the most appropriate substrate for differentiation into mature RPEs, which can benefit AMD patients for clinical treatment.

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Abstract **#982**

PROSTAGLANDIN E2 INDUCES SKIN AGING BY E-PROSTANOID 1 RECEPTOR IN NORMAL HUMAN DERMAL STEM/PROGENITOR CELLS

JOONG HYUN SHIM[P][1]

[1] *Faculty of Cosmetics and Beauty biotechnology, Semyung University*

Corresponding author's email: jhshim@semyung.ac.kr

The production of collagen type I is decreased during aging, leading to skin wrinkle and impaired function. PGE2, a lipid derived signaling molecule derived from arachidonic acid by cyclo-oxygenase, inhibits collagen production and induces MMP1 expressions by fibroblasts in vitro. The inhibition of collagen expressions and the promotion of MMP1 by PGE2 have been accepted as the aging mechanisms. I demonstrated that EP1 plays an important role in PGE2 signaling in normal human dermal stem/progenitor cells (hDSPCs). When EP1 expression was suppressed with EP1 siRNA, there were no significant changes in mRNA levels of COL1A1/MMP1 between siRNA-transfected hDSPCs and siRNA-transfected hDSPCs with PGE2. These results show that EP1 is a receptor for PGE2. The phosphorylation of ERK1/2 after PGE2 treatment significantly increased. In addition, the exposure of hDSPCs to PGE2 triggered an increase in intracellular Ca2+ concentrations. These results means that the PGE2 is directly associated with the EP1 receptor pathway-regulated ERK1/2 and IP3 signaling in hDSPCs.

Keywords

PGE2; EP1; human dermal stem/progenitor cells

Abstract **#983**

UTILIZATION OF IMMUNE CELLS TO AID IN BONE REGENERATION

Yang Zhang$_P$[1], Zhumei Zhuang[2], Abdullah Faqeer[1], Jeroen J.J.P. van den Beucken[3], Huanan Wang[2]

1 Shenzhen University

2 Dalian University of Technology

3 Radboudumc

Corresponding author's email: yang.zhang@szu.edu.cn

During bone regeneration, innate immune cells not only directly differentiate into osteoclasts to get involved in bone remodeling, but also affect the osteoblastic cell recruitment, migration, and osteogenic differentiation to establish bone formation. This information encourages us to consider immune cell implementation for bone regeneration. On the one hand, immune cells are plastic and sensitive to the local environment1, which provides the potential to modulate the microenvironment to manipulate the immune system in a favorable manner for enhanced bone repair and regeneration. On the other hand, due to the easy isolation procedure and high yield of immune cells2, immune cells have great practical potential to be directly utilized in cell-based approaches given reduced cost and (absence or short time of) in vitro expansion procedures.

In view of this, we investigated the potential to modulate the immune microenvironment by material properties such as roughness and stiffness and by particular cells such as MSCs. Both titanium surface roughness and hydrogel stiffness with or without MSCs positively affected the surrounding immune cell composition and cell phenotype. More importantly, we elucidated the function of macrophages and osteoclasts in bone formation and explored to promote osteogenic differentiation and bone formation by co-culturing and co-seeding MSCs with these immune cells. We also demonstrated that osteoclasts rather than MSCs or macrophages initiated the bone regeneration process, which offered subversive insights into functions of osteoclasts in the biological context of bone regeneration and opens up a new dimension for designing and preparing cell-based constructs for bone regenerative treatment.

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Abstract **#985**

DESIGN AND CHARACTERIZATION OF BIOINKS FOR EXTRUSION-BASED 3D BIOFABRICATION

Ugo D'Amora[1], Alfredo Ronca[1], Alessandra Soriente[1], Maria Grazia Raucci[1], Luigi Ambrosio[1]
[1] *Institute of Polymers, Composites and Biomaterials – National Research Council (IPCB-CNR)*
Corresponding author's email: ugo.damora@cnr.it

INTRODUCTION: Over the past years, biofabrication techniques offer the potential to produce structurally-ordered tissues with new and interesting approaches [1]. Different natural polymers have been studied as bioink for additive manufacturing. Bioinks should be able to be extruded through a thin needle, to retain a three-dimensional (3D) structure and to create a cytocompatible environment. However, their high solubility, high degradation rate and low mechanical properties limit their use. Different chemical strategies have been pursued to endow these materials with a shear thinning behaviour and improve the mechanical properties. In this study, gelatin and gellan-gum were chemically-modified and bioprinted to produce 3D scaffolds.

METHODS: Gelatin and gellan-gum were methacrylated to obtain GEMA and GGMA, respectively. The bioinks were characterized in terms of physico-chemical and rheological behaviour. 3D scaffolds were obtained by processing GGMA. Double network scaffolds were produced by combining GGMA structures with GEMA and alginate. Morphological, mechanical and biological performances were evaluated.

RESULTS: Scanning Electron Microscopy highlighted scaffolds were able to retain their well-ordered structure without collapse in the Z direction. Both kinds of structures showed improved mechanical properties. Furthermore, confocal images demonstrated cells initially adhered as agglomerates and then a spreading was observed.

CONCLUSIONS: The obtained results demonstrated the potential use of these bioinks for tissue engineering applications.

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Keywords
Bioink; Biofabrication; Hydrogel

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Abstract **#986**

COMPREHENSIVE LABEL-FREE CHARACTERIZATION OF EXTRACELLULAR VESICLES AND THEIR SURFACE PROTEINS

Eleni Priglinger[P][1,2], Jürgen Strasser[3,2], Boris Buchroithner[3,2], Florian Weber[3,2], Susanne Wolbank[1,2], Daniela Auer[4,5], Eva Grasmann[6], Claudia Arzt[6], Marie-Sophie Narzt[1,2], Johannes Grillari[1,7,2], Johannes Preiner[3,2], Jaroslaw Jacak[3,2], Mario Gimona[4,5,2,6]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Linz/Vienna, Austria*

[2] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[3] *School of Medical Engineering and Applied Social Science, University of Applied Sciences Upper Austria, Linz, Austria*

[4] *GMP Unit, Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria*

[5] *Research Program "Nanovesicular Therapies", Paracelsus Medical University, Salzburg, Austria*

[6] *Transfer Center for Extracellular Vesicles Theralytic Technologies (EV-TT), Paracelsus Medical University, Salzburg, Austria*

[7] *Institute of Molecular Biotechnology, Dept. of Biotechnology, BOKU – University of Natural Resources and Life Sciences, Vienna, Austria*

Corresponding author's email: eleni.priglinger@trauma.lbg.ac.at

Interest in mesenchymal stem cell derived extracellular vesicles (MSC-EVs) as therapeutic agents has dramatically increased over the last decade. Preclinical studies show that MSC-EVs have anti-apoptotic and neuroprotective effects, boost wound healing, and improve the integration of allogeneic grafts through immunomodulation. Current approaches to the characterization and quality control of EV-based therapeutics include particle tracking techniques, Western blotting, and advanced cytometry, but standardized methods are lacking. In this study, we established and verified quartz crystal microbalance (QCM) as highly sensitive label-free immunosensing technique for characterizing clinically approved umbilical cord MSC-EVs enriched by tangential flow filtration and ultracentrifugation. Using QCM in conjunction with common characterization methods, we were able to specifically detect EVs via EV (CD9, CD63, CD81) and MSC (CD44, CD49e, CD73) markers and gauge their prevalence. Additionally, we characterized the topography and elasticity of these EVs by atomic force microscopy (AFM), enabling us to distinguish between EVs and non-vesicular particles (NVPs) in a therapeutic formulation. This measurement modality makes it possible to identify EV sub-fractions, discriminate between EVs and NVPs, and to characterize EV surface proteins, all with minimal sample preparation and using label-free measurement devices with low barriers of entry for labs looking to widen their spectrum of characterization techniques. Our combination of QCM with impedance measurement (QCM-I) and AFM measurements provides a robust multi-marker approach to the characterization of clinically approved EV formulations and opens the door to improved quality control.

Abstract **#987**

THERMORESPONSIVE INJECTABLE HYDROGEL FOR DELIVERY OF NOTOCHORDAL CELLS FOR INTERVERTEBRAL DISC REGENERATION

Rebecca Williams[P][1], Shay Basatvat[1], Abbie Binch[1], Marianna Tryfonidou[2], Chris Sammon[1], Joseph Snuggs[1], Christine Le Maitre[1]

[1] *Sheffield Hallam University*

[2] *Utrecht University*

Corresponding author's email: b9032450@my.shu.ac.uk

Introduction

Low back pain is strongly associated with degeneration of the intervertebral disc (IVD). During IVD disc degeneration the central nucleus pulposus (NP) is broken down, due to altered matrix synthesis, increased degradation and cell. It has been proposed that the reinjection of Notochordal (NC) cells, are thought to have substantial therapeutic effects in mediating disc regeneration and restoring disc biomechanics. This study investigates the characteristic and behavioural changes of NC cells when cultured over a two-week period within our pNIPAM hydrogel system; otherwise known as NPgel.

Methods

Notochordal cells were extracted from porcine IVDs and incorporated into NPgel. The NC cell and NPgel constructs were cultured for up to two weeks under 21% and 5% oxygen. Histological, immunohistochemical and scanning electron microscopy (SEM) analysis was performed to investigate NC cell viability and altered matrix synthesis.

Results

SEM analysis revealed that NC cells can survive in the NPgel after two weeks under 5% oxygen. Additionally, from analysing the characteristics and phenotype of NC cells, we have found they survive in clusters. Further to this we will determine the expression of NC markers.

Conclusion

NC cells maintain their phenotype in vitro when encapsulated into our novel NPgel scaffold. The delivery of NC cells within NPgel into the degenerate IVD has potential for IVD regeneration.

Abstract **#988**

MAGNETIC RESPONSIVE PVDF MICROSPHERES FOR CELL CULTURE UNDER ELECTRO-MECHANICAL STIMULATION

José Luis Gómez Ribelles[1,2], Luis A Martins$_P$[1], Daniel Salazar-Jaramillo[3], Vanesa F Cardoso[4,5], Senentxu Lanceros-Méndez[3,6]

[1] *CBIT – Centre for Biomaterials and Tissue Engineering, Universitat Politècnica de València, 46022 Valencia, Spain*

[2] *Biomedical Research Networking Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Valencia, Spain*

[3] *BCMaterials, Basque Center for Materials Applications and Nanostructures, UPV/EHU Science Park, 48940 Leioa, Spain*

[4] *Center/Department of Physics, Universidade do Minho, 4710-057 Braga, Portugal*

[5] *MEMS—Microelectromechanical Systems Research Unit, Universidade do Minho, 4800-058 Guimarães, Portugal*

[6] *IKERBASQUE, Basque Foundation for Science, 48013 Bilbao, Spain*

Corresponding author's email: luisamaromartins@gmail.com

The composite consisting of a Polyvinylidene fluoride (PVDF) matrix with cobalt ferrites (CFO) nanofiller is one of the most common and effective polymeric magnetoelectric systems. The aim of this work was to produce this composite in the form of microspheres that could be applied for the electro-mechanical stimulation of cells in culture. Microfluidics offer the possibility of high reproducibility and size tunability. A flow-focusing polydimethylsiloxane (PDMS) device was fabricated based on a 3D printed polylactic acid (PLA) master. The device was used to produce microspheres between 79 and 330µm. To obtain an electric response under a magnetic field, processing PVDF into electroactive beta-phase is a mandatory condition for its electroactive behavior. Since processing takes place at a temperature below 60ºC beta-phase crystallization is favored (75%) avoiding the need, time and hassle of post-processing thus producing magnetoelectric microspheres in a one-step approach. Magnetic properties and high encapsulation efficacy of 80% were confirmed by vibrating-sample-magnetometry (VSM). Microstructure and nanoparticle distribution was assessed by scanning electron microscopy (FESEM) and through focused-ion-beam (FIB). Acknowledgements: This work has been funded by the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERFD) through the projects PID2019-106099RB-C41 and –C43 / AEI / 10.13039/501100011033.

Abstract **#991**

HETEROGENEITY IN LIPID AND PROTEIN CARTILAGE PROFILES ASSOCIATED WITH HUMAN OSTEOARTHRITIS WITH OR WITHOUT TYPE 2 DIABETES MELLITUS

Berta Cillero-Pastor[P][1], Maxime Eveque-Mourroux[1], Pieter Emans[2], Annelies Boonen[3], Britt Claes[1], Freek Bouwman[4], Ron Heeren[1]

[1] Maastricht MultiModal Molecular Imaging (M4I) institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, NL

[2] Maastricht University Medical Center, Department of Orthopedic Surgery, Maastricht, NL

[3] Maastricht University Medical Center, Department of Internal Medicine, Division of Rheumatology, and Care and Public Health Research Institute (CAPHRI), Maastricht, NL

[4] Maastricht University Medical Center, Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht, NL

Corresponding author's email: b.cilleropastor@maastrichtuniversity.nl

Background: Osteoarthritis (OA) is a multifactorial pathology and comprises a wide range of distinct phenotypes (1). In this context, the characterization of the different molecular profiles associated with each phenotype can improve the classification of OA for better therapies. In particular, OA can co-exist with type 2 diabetes mellitus (T2DM). This study investigates lipidomic and proteomic differences between human OA/T2DM- and OA/T2DM+ cartilage through a multimodal mass spectrometry approach.

Methods used: Human cartilage samples were obtained after total knee replacement from OA/T2DM- (n=10) and OA/T2DM+ patients (n=10). Label-free proteomics was employed to study differences in protein abundance and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) for spatially resolved-lipid analysis.

Results/central findings: Label-free proteomic analysis showed differences between OA/T2DM- and OA/T2DM+ phenotypes in several metabolic pathways such as lipid regulation. Interestingly, the phospholipase A2 protein was found overexpressed within the OA/T2DM+ cohort. In addition, MALDI-MSI experiments revealed that phosphatidylcholine and sphingomyelin species were characteristic of the OA/T2DM- group whereas lysolipids were more characteristic of the OA/T2DM+ phenotype. The data also pointed out differences in phospholipid content between superficial and deep layers of the cartilage.

Conclusion: Our study shows distinctively different lipid and protein profiles between OA/T2DM- and OA/T2DM+ human cartilage, demonstrating the importance of sub-classification of the OA disease for better personalized treatments.

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Abstract **#993**

HYPOIMMUNOGENIC HUMAN INDUCED PLURIPOTENT STEM CELLS PREPARED FROM AMNIOTIC FLUID STEM CELLS

Jhe-Yu Hsu$_P$[1], Akon Higuchi[1,2], Tzu-Cheng Sung[1], Yi-Peng Jiang[1]

[1] *Department of Chemical & Materials Eng., National Central University, Taoyuan, Taiwan*

[2] *Center for Emergent Matter Science, Riken, Saitama, Japan*

Corresponding author's email: hsujheyu1120@gmail.com

Human pluripotent stem cells (hPSCs) are a promising source of cells for tissue regeneration. However, histocompatibility of the transplanted cells remains a major challenge to their clinical application where human leukocyte antigen class (HLA) Ia (HLA-A, -B, -C) molecules are the primary mediators of immune reaction. There are many alleles in the HLA genes. Therefore, it is extremely hard to match the same HLA expressions from two different people. It is necessary to establish patient-specific stem cell line or stem cells bank having specific human leukocyte antigen (HLA) to avoid immune response of the patients.

Universal induced pluripotent stem cells (hiPSCs) were established without gene editing, which were reprogrammed from human amniotic fluid stem cells with mixing two allogenic donors; these cells do not or less express human leukocyte antigen (HLA) class Ia (HLA-A, -B, and -C) and class II even after differentiation into cardiomyocytes, embryoid bodies (progenitor cells derived from three germ layers), and mesenchymal stem cells (universal hiPSCs). Cardiomyocytes differentiated from universal hiPSCs survived and continued beating even after treatment with allogenic mononuclear cells derived from different amniotic fluid (AF) donors.

We consider that the mechanism underlying the generation of universal hiPSCs is that fetal stem cells contain a specific group of cells that do not express HLA class Ia and class II but may express HLA-G, PD-L1 and/or CD47, which are expressed in baby tissue to avoid immune rejection with her mother, after the cells are reprogrammed into hiPSCs and subsequently differentiated into tissue cells.

Abstract **#995**

TREATING OBESITY BY ENHANCING FAT THERMOGENESIS WITH NON-VIRAL GENE DELIVERY OF VEGF

Ramyar Chavoshinejad$_P$[1], Michael E Symonds[2], James E. Dixon[1]

[1] *Division of Regenerative Medicine and Cellular Therapies, School of Pharmacy, University of Nottingham, UK*

[2] *Division of Child Health, Obstetrics and Gynaecology, School of Medicine, University of Nottingham, UK*

Corresponding author's email: ramyar.chavoshinejad@nottingham.ac.uk

Obesity, in which excess body fat accumulates, is the leading preventable cause of death. Metabolic syndrome, with comorbidities such as type-2 diabetes, high blood pressure, and circulating cholesterol and triglyceride also increases the negative effect of excess adipose tissue on health. The browning of white adipocytes would be an innovative intervention to treat these types of metabolic disorders.

We are pioneering the use of non-viral gene therapy to 'convert' white adipose tissue (WAT) to a beige/brown thermogenic, 'energy burning' phenotype. Previous studies have shown that enhanced Vascular endothelial growth factor (VEGF) activity in WAT can induce beige characteristics (Mitochondria and Uncoupling protein-1 (UCP1) abundance). We are therefore adapting our glycosaminoglycan (GAG) binding enhanced transduction (GET) gene therapy system for safe programming of fat to beige characteristics either by in situ delivery or modified autografts. Initially, we have developed a tractable model for adipogenesis, and transdifferentiation to test GET-VEGF gene delivery using human mesenchymal stem cells (MSCs), which can be successfully differentiated to both types of mature adipocytes. Presently, we are testing the influence of known factors to induce beiging, such as cold or caffeine compared to VEGF gene delivery. We characterise the adipocytes generated with immunostaining, gene expression analyses, and mitochondrial seahorse assay to assess their phenotype and thermogenic potential.

Improvements in diet, and exercise are of course necessary to reduce the obesity-related disease epidemic. However, regenerative medicine strategies through patient cell programming could promote transition to balanced metabolism and treat one of the most presentable causes of early death.

Keywords

Obesity; VEGF; GET

Abstract **#996**

BIOACTIVE MEMBRANES FOR THE FUTURE TREATMENT OF OSTEOPOROTIC FRACTURES.

Ana Sofia Silva$_P$[1], Lúcia Ferreira Santos[1], Ana Lúcia Cunha[1], Sara Nadine[1], Clara Rosa Correia[1], João Filipe Mano[1]
[1] *CICECO - Aveiro Institute of Materials, University of Aveiro*
Corresponding author's email: sofiamsilva@ua.pt

Fragility fractures are the main consequence of osteoporosis and their treatment remains a challenge in the orthopedic field. Evidences demonstrate that an impaired periosteal activity is responsible for recurrent fractures. Hence, we suggested the development of a natural-based regenerative membrane fixing biological active capsules for periosteum regeneration purposes. A laminarin hydrogel was microfabricated with suitable mechanical properties and adequate resorbable times for bone regeneration. The membrane acted as on-site fixing agent for biological active capsules and is expected to be implanted by wrapping the membrane around the defect to guide bone regeneration. Alginate liquified capsules were produced via electrohydrodynamic spraying,[1,2] and comprised i) microparticles for cell adhesion and for the transport of osteogenic-differentiating factors, and ii) a cell niche of ASCs and HUVECs. Then, layer-by-layer was performed using 3 different polyelectrolytes until a 10-layered membrane was created. Methacrylated laminarin (MeLam) hydrogels were produced by bringing a solution of MeLam in contact with an optimized PDMS master, followed by UV irradiation. [3] Capsules with optimized sizes were entrapped within the micropillars of the membrane achieving higher entrapments when capsules diameters matched the micropillars spacing. In vitro osteo- and angiogenic potential was assessed in the individual components and after their conjugation (membrane+capsules). The bioactive membrane was also placed in contact with a fibroblast cell monolayer where membrane integration was achieved. In brief this bioactive membrane is expected to induce regional bone formation and an overall stimulation of bone regeneration.
We acknowledge ATLAS: ERC-2014-ADG-669858, PROMENADE: PTDC/BTM-MAT/29830/2017 and CICECO-Aveiro Institute of Materials: POCI-01-0145-FEDER-007679 (UID/CTM/50011/2013).

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Abstract **#997**

THE IMPACT OF SUPERCRITICAL CARBON DIOXIDE ON THE BIOLOGICAL AND BIOMECHANICAL PROPERTIES OF DECELLULARISED HEART VALVES

Sahirah Aslam[P][1,2], Amisha Desai[1,3], Paul Rooney[4], Jennifer H Edwards[1,2,3], Louise M Jennings[1,3], Helen E Berry[1,2]

[1] *Institute of Medical and Biological Engineering, University of Leeds, UK*

[2] *School of Biomedical Sciences, University of Leeds, UK*

[3] *School of Mechanical Engineering, University of Leeds, UK*

[4] *NHS Blood and Transplant, UK*

Corresponding author's email: bssa@leeds.ac.uk

Decellularised heart valves are a promising option for heart valve replacement. A terminal sterilisation method is required to enhance the clinical translation of these novel decellularised biological scaffolds and assure patient safety. Supercritical carbon dioxide (ScCO2) is a robust sterilisation technique that is ideal for the application on biomaterials [1]. Within this study, the impact of ScCO2 sterilisation, supplemented with oxidising agents, on the biological and biomechanical properties of decellularised pulmonary heart valves (PHVs) was explored. There were no observational differences within the histoarchitecture and the microscopic structures of the ScCO2 treated PHVs in comparison to decellularised PHVs, and no reduction in the collagen content. There was, however, a significant reduction in the thermal stability of the ScCO2 treated PHV tissue, significant increase in the elastin phase slope and ultimate tensile strength biomechanical parameters, and poor cytocompatibility. The presence of residual oxidising agents which may be responsible for the cytotoxicity, which may be avoided by rinsing the tissue graft prior to in vitro testing [2]. Therefore, this study concludes that ScCO2 is still a promising sterilisation option for the application on soft tissues.

Keywords

Pulmonary Heart Valve ; Sterilisation ; Supercritical Carbon Dioxide

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Abstract **#998**

ADAPTABLE HYDROGELS FOR ORGANOID CULTURE

Sarah Heilshorn[P][1]

[1]*Stanford University*

Corresponding author's email: heilshorn@stanford.edu

While organoid culture has the potential to revolutionize our understanding of human biology, current protocols rely on the use of Matrigel, a complex, heterogeneous material with large batch-to-batch variations that hinder reproducibility. In response, several groups have begun designing synthetic hydrogel systems to enable the reproducible culture of organoids. Recently, the matrix stress relaxation rate (i.e. the ability of a hydrogel to remodel its network connectivity in response to an applied stress) has been demonstrated to have profound effects on encapsulated cells. To date, the role of matrix stress relaxation on organoid cultures has not been explored. Here we present the design of a family of double-network hydrogels that undergo two stages of crosslinking: the first stage uses reversibly dynamic covalent chemistry bonds, while the second stage reinforces the hydrogel through thermal-induced polymer aggregation. This double-network of physical interactions results in a gel a broad dynamic range of tunable mechanical properties, where the gel stiffness is set by the number of crosslinks and the gel stress relaxation rate is independently set by the kinetics of the crosslink binding and unbinding. These novel, double-network hydrogels have been used to study the role of mechanotransduction in the culture of patient-derived, human intestinal organoids. In this system, we find that the organoid cultures display strong phenotypic responses to matrix stress relaxation, which are dependent on cell-matrix interactions with both CD44 and integrin cell-surface receptors.

Keywords

organoids

Abstract **#999**

A THREE-DIMENSIONAL MECHANICAL LOADING MODEL OF HUMAN OSTEOCYTES IN THEIR NATIVE MATRIX TO STUDY METABOLIC BONE DISEASE

Chen Zhang$_P$[1,2], Elisabet Farré-Guasch[1], Jianfeng Jin[1], Huib W. van Essen[2], Jenneke Klein-Nulend[1], Nathalie Bravenboer[2]

[1] *Academic Centre for Dentistry Amsterdam, University of Amsterdam and Vrije Universiteit Amsterdam, Dept Oral Cell Biology, Amsterdam Movement Sciences, Amsterdam, The Netherlands*

[2] *Amsterdam University Medical Centers/location VUmc, Vrije Universiteit Amsterdam, Dept Clinical Chemistry, Amsterdam Movement Sciences, Amsterdam, The Netherlands*

Corresponding author's email: c.zhang@acta.nl

The mechanosensitivity of osteocytes is affected by their microenvironment, which is changed in metabolic bone disease. However, the native calcified matrix makes the study of osteocytes technically difficult. We aimed to develop a 3D-human osteocyte model to study mechanosensitivity of osteocytes in their native matrix under physiological or pathological loading. Osteocyte viability, osteocyte-specific gene expression, and microdamage under well-defined loading magnitudes was determined.

Small human bone pieces (8x3x1.5 mm; surgical waste) were physiologically (1000 -3000 με) or pathologically (>3000 με) loaded for 5 min by sinusoidal displacement using a micromover. Osteocyte viability was quantified 24 h after loading by LDH staining. Loading-induced microdamage was quantified in BaSO4-stained bone using micro-computed tomography. Besides mechanoresponsiveness, osteocyte responsiveness to 10-7 M 1,25-dihydroxyvitamin D3 for 24 h was assessed.

Loading magnitude (2302-13811 με) or micromover displacement and reaction force exerted by bone were linearly related. Osteocyte viability was not affected by physiological mechanical loading, while pathological loading caused cell death. Microdamage was increasing with increased loading magnitude (2500-7500 με). Osteocytes were responsive to 1,25(OH)2D3, i.e. GALNT3 was downregulated, while CYP24, as well as E11, SOST, and FGF23 gene expression were upregulated.

In conclusion, we developed a 3D-mechanical loading model of osteocytes in their native matrix that allows the application of defined physiological loading magnitudes to human bone while maintaining cell viability without causing microdamage. This model would be useful for studies on osteocyte mechanosensitivity in human metabolic bone disease.

Keywords

Osteocyte; Native matrix; Mechanical loading

Abstract **#1000**

MATERIAL-DRIVEN FIBRONECTIN AND LAMININ NETWORKS FOR EFFICIENT GROWTH FACTOR PRESENTATION TO BIOENGINEER BONE MARROW NICHE IN VITRO

Yinbo Xiao[P][1], Hannah Donnelly[1], Dominic Meek[2], Manuel Salmeron-Sanchez[3], Matthew J. Dalby[1]

[1] *Centre for the Cellular Microenvironment, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK.*

[2] *Department of Orthopedics, Queen Elizabeth II University Hospital, Glasgow G51 4TF, UK.*

[3] *Centre for the Cellular Microenvironment, Division of Biomedical Engineering, School of Engineering, University of Glasgow, Glasgow G12 8LT, UK.*

Corresponding author's email: y.xiao.1@research.gla.ac.uk

Mesenchymal stem cells (MSCs) are well known to hold great potential to support hematopoietic stem cell (HSC) growth and self-renewal. However, when isolated from their specialized microenvironment in vivo, termed the stem cell niche, both MSCs and HSCs lose their regenerative capacity and niche phenotype. The niche provides both physical and functional regulatory cues that control cell-intrinsic and cell-extrinsic processes, which is a major challenge to be recapitulated in culture. Here, using poly (ethyl acrylate) (PEA) polymers, we develop a high-throughput platform to closely represent main compartments of the bone marrow niche and dissect their roles on MSC fates simultaneously. PEA coated surfaces promote spontaneous unfolding of the ECM proteins Fibronectin (FN) and Laminin (LM), leading to physiological-like nanonetwork formation that exposes key cell- and growth factor (GF)- binding domains. The GF binding domain allows the presentation of ultralow doses of niche GFs, such as bone morphogenetic protein 2 (BMP-2), nerve growth factor (NGF) and C-X-C motif chemokine 12 (CXCL12) to cells alongside a synergistic integrin-binding site1,2. Using this novel high-throughput platform, we assess the combined effects of ECM, GFs as well as hypoxia/normoxia on MSCs behaviours, unveiling the optimal niche conditions to direct MSCs towards HSC-support phenotype in culture. This material-based system provides a high throughput approach to rapidly and efficiently test multiple combinations of biochemical and mechanical niche cues on cell fate regulation. Such novel improved niche-like model could be further utilized to investigate the fundamental mechanisms that control self-renewal in both MSCs and HSCs in the niche.

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Abstract **#1001**

ISOLATION OF PRIMARY HUMAN COLON TUMOR CELLS FROM SURGICAL TISSUES BY MEMBRANE FILTRATION METHOD

JIA HUA WANG$_P$[1], WEI LUN HUANG[1], YI PENG JIANG[1], Akon Higuchi[2]

[1] *Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli, Taoyuan 32001, Taiwan*

[2] *Riken Cluster for Pioneering Research, RIKEN, Wako, Saitama 351-0198, Japan*

Corresponding author's email: a0922416954@gmail.com

Cancer stem cells (CSCs, cancer-initiating cells) are responsible for cancer proliferation and show stronger resistance than other cancer cells under conventional cancer therapy. Precision medicine shows positive effect for cancer patients treatment. Therefore, isolation of patient specific primary cancer cell line is important for precision medicine screening. However, CSCs typically comprise 1%–5% of the total tumor cell population. It is hard to distinguish CSCs from other cells in primary tissue. Thus, we have been developing a membrane filtration method to target CSCs and establish primary colon cancer cell line.

This membrane filtration method was performed via Nylon mesh filter or poly(lactic-co-glycolic acid)-silk screen membranes. The primary colon cancer cells were isolated by several steps including washing, mincing and digesting and the cancer cell solution was permeated through these membranes. CSCs and colon cancer cells were characterized using CSC surface marker expression, colony forming assay and expression of carcinoembryonic antigen (CEA) using ELISA assay. We also analyzed colon cancer cell lines using this method as a model of primary colon cancer tissue solution.

This purification method using membrane filtration method has been successfully verified using Lovo cancer cell line and the cells showing high level expression of CSCs marker (CD133, CD44) were isolated where CSC markers were analyzed using flow cytometry. We expect to establish patient specific colon cancer cell line by the membrane filtration method with different pore size, affinity and biocompatibility. This method is a promising approach, which can be applied for precision medicine screening in the future.

Abstract **#1003**

DYNAMIC IN VITRO LONG-TERM CULTIVATION OF A MULTI-CELLULAR TUBULAR BONE MODEL TO INVESTIGATE THE BIOCOMPATIBILITY OF NEW BONE IMPLANTS

Dierk Gruhnᴘ[1], Richard Viebahn[2], Jochen Salber[1,2]

[1] *Salber Laboratory, Centre for Clinical Research, Department of Experimental Surgery, Ruhr-Universität Bochum, Bochum, Germany*

[2] *Department of Surgery, Universitätsklinikum Knappschaftskrankenhaus Bochum GmbH, Bochum, Germany*

Corresponding author's email: jochen.salber@ruhr-uni-bochum.de

It was our aim to develop human tubular bone models to simulate the structural composition and physiological processes as closely as possible to those in natural bone. Taking into account the complexity of natural mammalian bone, we followed a consecutive bottom-up assembly approach using directional electrospinning and drop on demand bio-printing. To mimick bone homeostasis we focused on custom-made bioinks containing stimulated monocytes (hMoCD14+-PD) to form osteoclasts and hMSC derived osteoblasts. 3D bone mimicking constructs were produced by alternating drop on demand layers and pre-cultured for three weeks. Bilayered periosteum was prepared by directional electrospinning of a growth factors enriched medical grade biopolymer. Afterwards fibroblasts and endothelial cells were co-cultered in the bilayered scaffold for two weeks. Assembled multi-cellular tubular constructs were cultivated in a custom-made bioreactor using minimal medium for up to two month. The constructs were IHC stained and analyzed. Mikro-CT over time, SEM-EDX and TEM examinations of the bony constructs were performed. After complete structural, cellular and biochemical characterization of this tubular bone, custom-made 24-well plates were loaded with these specimen, biomaterials to be tested were implanted and then dynamically cultivated for different time periods. To assess in vitro biocompatibility, a comprehensive panel of analyses was used. With the help of this model, significantly fewer animal experiments will be required in the future for the preclinical evaluation of new implant materials, designs or modifications.

This project has received funding from the European Union's Horizon 2020 research and innovation action under grant agreement No. 814495-EVPRO (www.evpro-implant.eu).

Abstract **#1004**

GENETIC SIGNATURE AND BIOMECHANICAL CHARACTERIZATION OF NATIVE ENTHESES IN A RAT MODEL

Carlos Peniche Silva[P][1], Sebastian A. Müller[2], Damian van der Broeck[3], Nicholas P. Quirk[2], Rodolfo E. De la Vega[1,2], Christopher H Evans[2], Martijn van Griensven[1,2], Elizabeth R. Balmayor[2,3]

[1] *CBITE, MERLN Institute for Technology - Inspired Regenerative Medicine, Maastricht University, The Netherlands*

[2] *Rehabilitation Medicine Research Center, Mayo Clinic, Rochester, Minnesota, USA*

[3] *IBE, MERLN Institute for Technology - Inspired Regenerative Medicine, Maastricht University, The Netherlands*

Corresponding author's email: c.penichesilva@maastrichtuniversity.nl

Tissue engineers have been working to develop strategies for enthesis regeneration. The patellar (PT), Achilles (AT), and Rotator cuff (RC) tendons are the most relevant sites of injury. We aimed to characterize their entheses concerning genetic signature and biomechanics in rats. RC showed the highest tangent modulus and lowest ultimate strain. This relationship was inverse for AT. PT showed always an intermediate value for both properties. The gene signature was studied for >150 ECM and osteogenic genes. Clear differences were observed. AT showed an upregulation of relevant genes compared to the PT and RC entheses, including collagens (Col I, II, IV, V), BMPs, cadherins, and metalloproteinases. This correlates with the highest ultimate strain obtained for the AT. RC enthesis yielded the lowest overall expression of ECM genes except for the upregulation of Col I and tenascin-C, while PT showed a strong downregulation of osteogenic genes compared to AT and RC. The expression levels for scleraxis and tenomodulin followed the order RC>AT>PT and AT>PT>RC, respectively. The expression of tenomodulin was higher than scleraxis for all the entheses. Interestingly, PT showed the highest difference between tenomodulin and scleraxis expression. Array-based gene expression was validated by qRT-PCR for relevant genes. The data collected here provide valuable insights into the genetic profile of the enthesis in three different anatomic localizations, and their correlation with biomechanical properties. Our results indicate the PT enthesis as optimal localization for an in vivo regeneration model. Furthermore, these data highlight relevant genes that must be stimulated to achieve regeneration.

Abstract **#1005**

TRI-LAYERED CONSTRUCTS FOR CORONARY ARTERY REGENERATION

Kenny van Kampen[1], Rebeca Rivero[1], Carlos Mota[1], Matthew Baker[1], Lorenzo Moroni[1]

[1] *MERLN Institute, Maastricht University*

Corresponding author's email: j.fernandezperez@maastrichtuniversity.nl

Coronary diseases affect millions worldwide and are commonly treated with a bypass using autologous tissue, usually from the saphenous vein or the internal thoracic artery. These procedures may induce secondary site morbidity and are not always possible due to insufficient tissue availability. Currently developed tissue engineered grafts rely on lengthy culture periods, which are costly and not always off the shelf. It is for these reasons that in situ tissue engineering approaches are gaining interest. These scaffolds are intended to be implanted acellularly and encourage the body to repopulate and remodel them over time.

In this study we have fabricated scaffolds by Additive Manufacturing of PCL onto a rotating mandrel. The tubular structures were produced with variety of fiber alignments by modifying the extruder speed and increased fiber roughness via sodium hydroxide etching. These scaffolds supported the adhesion and proliferation of HUVECs and hMSCs differentiated into vascular smooth muscle-like cells. Cells repopulating the media layer aligned along the fibers and laid their own extracellular matrix and the intima layer consisted of a fully endothelialized lumen. To improve the mechanical properties of these constructs, an external layer was added with sine auxetic patterns, which intend to replicate the crimped collagen in the vessel wall. Furthermore, a pro-angiogenic hydrogel based on supramolecular non-covalent binding of alginate was used to emulate the adventitia layer. Future work will focus on the introduction of bioactive cues to the scaffolds to promote endogenous cell recruitment upon implantation.

Funded by RegMedXB and Health~Holland.

Keywords

vascular graft

Abstract **#1007**

INTEGRATING MOLECULAR SELF-ASSEMBLY AND ADDITIVE MANUFACTURING FOR THE BIOFABRICATION OF MICROFLUIDIC DEVICES WITH BIOLOGICALLY RELEVANT PROPERTIES

Yuanhao Wu[P][1,2], Babatunde O Okesola[3], John Connelly[3], Richard O.C. Oreffo[4], Jose Carlos Rodriguez-Cabello[5], Giovanni Vozzi[6], Helena S. Azevedo[3], Nicola M. Pugno[7], Alvaro Mata[1,2]

[1] School of Pharmacy, University of Nottingham, UK

[2] Department of Chemical and Environmental Engineering, University of Nottingham, UK

[3] School of Engineering and Materials Science, Queen Mary University of London, London E1 4NS, UK

[4] Bone and Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton SO16 6YD, UK

[5] BIOFORGE Group, University of Valladolid, CIBER-BBN, 47011 Valladolid, Spain

[6] Research Center'E. Piaggio' & Dipartimento di Ingegneria dell'Informazione, University of Pisa, Largo Lucio Lazzarino, 256126 Pisa, Italy

[7] Laboratory of Bio-inspired, Bionic, Nano, Meta Materials & Mechanics, Università di Trento, via Mesiano, 77, I-38123 Trento, Italy

Corresponding author's email: Yuanhao.Wu@nottingham.ac.uk

Organs-on-chip offer the opportunity to recreate a variety of biological niches1. However, most materials used to fabricate these systems do not replicate the physical and chemical properties of the native environment. Self-assembly represents an attractive route to develop more biologically relevant materials2 with enhanced modularity, tuneability, and structural hierarchy. Here, we report an elastin-like protein (ELP)-graphene oxide (GO) self-assembling and biofabricated fluidic device with biologically relevant properties for organ-on-chip applications3.

This ELP-GO co-assembling system integrates advantages of self-assembly and additive manufacturing to develop capillary-based fluidic devices exhibiting a series of physiological chemical and physical properties such as no need of toxic cross-linker, stable for at least 1 month, and generating tubular networks down to 10 µm in internal diameter, 2 µm in wall thickness. The liquid-in-liquid process is cell-friendly and facilitates bioprinting, resulting in fluidic structures (assessed via in vitro and ex vivo experiments) that can withstand pulsatile flow immediately after bioprinting. Taking advantage of the self-assembling process, we demonstrate the capacity to tune capillary porosity and permeability by varying the content of GO percentage and cell density and demonstrate that the capillaries exhibit a Young Moduli ~500 kPa. We have also standardized the 3D printing process by optimizing the parameters of 3D printer.

The study introduces an innovative way to grow fluidic devices by self-assembly. This device may offer potential applications in the field of biomedicine such as tissue engineering scaffolds, microfluidic systems, and organ-on-chip devices.

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Abstract **#1008**

TRANSCRIPTOME ANALYSIS OF STRETCHED HUMAN OSTEOCYTIC CELLS REVEALS ACTIVATION OF SIGNALLING PATHWAYS IMPLICATED IN CELL CYCLE, DNA-REPAIR AND CANCER

Livia Santos$_P$[1,2,3], Aslihan Ugun-Klusek[4,2,3], Clare Coveney[5,2,3], David Boocock[5,2,3]

[1] *Department of Sport Science*

[2] *School of Science and Technology*

[3] *Nottingham Trent University*

[4] *Department of Biosciences*

[5] *John van Geest Cancer Research Centre*

Corresponding author's email: livia.santos@ntu.ac.uk

Introduction: Regenerative rehabilitation is an emerging area of regenerative medicine that is raising interest in cancer and orthopaedics. It was recently discovered that mechanically stimulated osteocytes appear to protect the bone against breast cancer metastasis (1). Aiming to further understand how osteocytes may protect bone from cancer, we decided to analyse the transcriptome of human and mouse osteocytic cells after a single bout of mechanical loading. Insights from this study could help the development of regenerative rehabilitation protocols aiming to avoid bone cancer progression.
Methods: The murine osteocytic cell line MLO-Y4 (Kerafast) was cultured according to the supplier instructions. Human stem cells (ATCC) were expanded and differentiated into osteocyte-like cells (hOC) following the manufacturer instructions. Cells were cultured in a computer-controlled bioreactor (Flexcell Int) under cyclic stretch (3.4%, 2Hz, 5h). Samples were analysed by RNA sequencing (Illumina NovaSeq 6000). Differential expression analysis between stretched cells and static control was performed using the DESeq2 R package.
Results: RNA-seq detected ~13,000 gene products in the murine cell line MLO-Y4 and ~14,000 in hOC. A comparative analysis across human and mouse osteocytic cell lines evidenced that the transcriptome was quite distinctive between the two. KEGG analysis of the human transcriptome identified as potential mechanosensitive signalling pathways, FoxO, cell cycle, DNA-repair and cancer, whereas examination of mouse identified, TNF, cytokine-cytokine receptor interactions, PI3K-AKT, Jak-STAT and HIF-1.
Conclusion: Several pathways related to cell cycle, DNA-repair and cancer were found significantly enriched in mechanically stimulated hOC. Functional studies are necessary to confirm a cancer protective effect.

Keywords

regenerative rehabilitation; osteocytes; cancer

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Abstract **#1009**

SURFACE FUNCTIONALISED BIOMATERIALS AND NANOSTRUCTURES FOR ADVANCED THERAPIES

Nuno M. Neves[P][1]

[1] *3B's Research Group/University of Minho*

Corresponding author's email: nevesi3bs@gmail.com

Many biomaterials were proposed to produce porous scaffolds, nanofibers and nanoparticles for different medical treatments and applications. Systems combining natural polymers and synthetic biodegradable polymers are particularly adequate for those demanding applications. Those biomaterial systems can be tailored with enhanced mechanical properties, processability, cell-friendly surfaces and tunable biodegradability. Our biomaterials may be processed by melting or solvent routes into devices with wide range of applications such as biodegradable scaffolds, films or particles and adaptable to many biomedical applications.

Non-woven meshes of polymeric ultrafine fibers with fiber diameters in the nanometer range can be produced by electrospinning. Those meshes are highly porous and have a high surface area-to-volume ratio and can mimic the structure of the extracellular matrix of human tissues and can be used as scaffolds for Tissue Engineering (TE). There is a great interest in developing also nanoparticles and hydrogels from those polymeric systems for injectable treatment modalities. All those structures can be used as substrates for specific surface functionalization having fine-tuned biological properties. This strategy enables developing highly controlled devices for exposure, capture and, if needed, inactivation of biological biomolecules relevant for novel treatment modalities in various disease conditions.

This talk will review our latest developments in biomaterials, nanoparticles and nanofibre meshes in the context of novel therapeutic applications.

Abstract **#1010**

EFFECTS OF DIFFERENT XENO-FREE BIOMATERIALS FOR MESENCHYMAL STEM CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Chun-Ko Wang[P][1], Ming-Wei Lu[1], Akon Higuchi[1,2,3]

[1] *Department of Chemical and Materials Engineering, National Central University, No. 300, Jhongda RD., Jhongli, Taoyuan 32001, Taiwan*

[2] *Riken Cluster for Pioneering Research, RIKEN, Wako, Saitama 351-0198, Japan*

[3] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*

Corresponding author's email: bestsho125@gmail.com

Human mesenchymal stem cells (hMSCs) are a promising cell source in clinical trials which can protect host tissues from graft-versus-host disease (GVHD). However, hMSC treatment faces limited passage numbers of hMSCs. The goal of this study is that we develop a suitable xeno-free condition protocol of human pluripotent stem cells-derived hMSCs (hPSC-MSCs) and screen the extracellular matrix (ECM) effect for finding appropriate ECM-coated surface for MSC differentiation. In this study, we modified the differentiation protocol [1] of hPSCs-MSCs by using inhibitors. We changed the protocol of the usage of ECM from xeno-containing Matrigel to five different xeno-free ECMs, including recombinant vitronectin, laminin-511, laminin-521, fibronectin, and collagen, and analyzed the characterization of MSCs from morphologies, MSC surface markers expression (CD44, CD73, CD90, and CD105), and tri-lineages differentiation.

We found that Laminin-521 and Collagen type I were the top two biomaterials for hPSCs differentiation into hMSCs. Comparing to the hPSC morphology, hPSC-MSCs have already become spindle shapes morphologies. The result of the cells cultured on these two conditions showed better double cells population and the highest MSC marker expression. Therefore, Laminin-521 and Collagen type I coated surfaces are considered to be the most reliable and xeno-free cell culture substrates for hPSC-hMSCs differentiation.

We are evaluating the differentiation ability of hPSC-MSCs into tri-lineage differentiation. We will further analyze the human leukocyte antigen (HLA) expression after hPSC-MSCs are treated with mononuclear cells to evaluate the protective effect from GVHD. We expect that our hPSC-MSCs can be widely applied for regenerative medicine in the future.

Keywords

Human mesenchymal stem cells; Human pluripotent stem cells; Biomaterial

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Abstract **#1011**

CULTURES AND CURES: NEURODIVERSITY AND BRAIN ORGANOIDS

Andrew J. Barnhart[P][1], Kris Dierickx[1]

[1] *Centre for Biomedical Ethics & Law KU Leuven*

Corresponding author's email: andrew.barnhart@kuleuven.be

Research with cerebral organoids is beginning to make significant progress in understanding the etiology of Autism Spectrum Disorder (ASD). Yet in doing so, researchers often incorporate the medical model of disability into the conceptual framework of their research, with a specific aim of potentially finding personalized treatments for individuals with ASD. The neurodiversity movement – a developmental disability movement that understands autism as a form of natural human diversity – will potentially disagree with approaches or aims of cerebral organoid research on ASD, since these advocates incorporate the social model of disability into their movement. Therefore, a potential conflict may arise between these perspectives over how to proceed with cerebral organoid research in regard to neurodevelopmental conditions. Here, we present these perspectives and give recommendations to achieve a more holistic and inclusive approach to cerebral organoid research on ASD.

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Abstract **#1012**

IRON NANOPARTICLE-LOADED HYDROGELS AS FUNCTIONAL COMPOSITE MATERIALS FOR 3D BIOFABRICATION

Christoph Rehbock[P][1], Yaya Li[1], Stephan Barcikowski[1]

[1] *Technical Chemistry I and Center for Nanointegration Duisburg-Essen, University of Duisburg-Essen, Essen, Germany*

Corresponding author's email: christoph.rehbock@uni-due.de

Nanocomposites constitute an interesting class of materials for tissue engineering , where the embedding of nanoparticles into a biocompatible polymer adds novel functionality and hence, improves viability and proliferation of relevant adhered cells. Our group has developed an elegant method for the fabrication of nanocomposites based on laser ablation in liquids in polymer and monomer solutions, yielding nanocomposites with outstanding purity and biocompatibility [1]. In previous works we could already demonstrate improved cytocompatibility of these materials through two separate pathways I) local changes in surface charge and stiffness [2] and II) Release of metal ions [3].

In our most recent work we focused on alginate and PEG hydrogel-based biomaterials loaded with iron nanoparticles. We examined the mechanism of metal ion release from these biomaterials and could find a significantly enhanced adhesion of model serum proteins to the biomaterial surface, which, interestingly already occur at very low nanoparticle loadings (< 0.1 wt%). This seems to indicate a mechanism based on locally elevated iron ion concentrations. [4] We further verified the suitability of these materials for 3D bioprinting applications where the nanoparticles had no adverse effects on printability. [3] [4] These findings were complemented by recent examinations with hematopoietic stem and progenitor cells in 2D and 3D culture. Here we found a significant influence of iron nanoparticles in the biomaterial on the early stage of in vitro erythropoiesis relevant for applications in in vitro blood farming.[5]

Keywords

iron nanoparticles; hydrogel; ion release

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Abstract **#1013**

CALCIUM AND ZINC RELEASING NANOPARTICLES FOR WOUND HEALING APPLICATIONS

Celia Ximenes-Carballo[P][1], Barbara Blanco-Fernandez[1], Soledad Pérez-Amodio[1,2,3], Oscar Castano-Linares[1,2,4], Elisabeth Engel[1,2,3]

[1] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain*

[2] *CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain*

[3] *Materials Science and Metallurgical Engineering, Polytechnical University of Catalonia (UPC), Barcelona, Spain*

[4] *Electronics and Biomedical Engineering, Universitat de Barcelona (UB), Barcelona, Spain*

Corresponding author's email: cximenes@ibecbarcelona.eu

Introduction: Chronic wounds represent a major burden in human society, with high costs associated with extensive care treatments. Research has focussed on the development of new wound healing devices. However, a device that enables fast-effective closure, low cost, and scalability is still missing.

Ions such as calcium and zinc are essential for skin homeostasis. Calcium regulates a plethora of skin vital functions. We have shown that calcium releasing platforms such as calcium phosphate nanoparticles (NPs) stimulate in vitro and in vivo wound healing [1,2]. On the other hand, zinc deficiencies are associated with impaired wound healing. zinc's antimicrobial properties make this ion a potential substitute for antibiotics.

This work aims to develop different ion releasing platforms based on nanocomposites for local and sustained ion release at the wound site.

Methods: NPs incorporating Zn2+ and Ca2+ were synthesized by double emulsification, nanoprecipitation and sol-gel synthesis. Ion release was measured by colorimetric methods. NPs were encapsulated in 3D printed gelatine scaffolds.

Results: NPs were synthesized with diameters ~300 nm and narrow size dispersity. Both ions were successfully incorporated into the carrier NPs. Scaffolds showed good particle distribution. The release of zinc ions showed enhanced antibacterial activity.

Discussion and Conclusion: Ion releasing platforms were successfully produced. Their composition, size, and ion release profiles indicate their potential use in soft tissue applications such as wound healing therapies.

Acknowledgments: This work was supported by the Spanish Ministry and the EuroNanomed3 program through the project nAngioDerm

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Abstract **#1014**

IN SITU DETECTION OF INFECTIONS IN BURN WOUNDS BY DIAGNOSTIC HYDROGEL WOUND DRESSINGS

Arn Mignon$_P$[1], Josefien Schietse[1], Manon Minsart[1], Sandra Van Vlierberghe[1], Peter Dubruel[1]

[1] Polymer Chemistry & Biomaterials Research Group, Centre of Macromolecular Chemistry (CMaC), Ghent University, Krijgslaan 281, S4 bis, 9000 Gent, Belgium

Corresponding author's email: arn.mignon@kuleuven.be

In Belgium, around 120 000 people suffer from burns yearly. Burns still remain a major clinical challenge, mainly due to bacterial infection. Nowadays, infections are diagnosed by removing the wound dressing followed by wound sampling and sample analysis. In situ detection of infection by means of a color signal can alert health care staff in an early state and improve clinical outcomes. In the current work, different acrylate endcapped urethane based precursors (AUP) were combined with diagnostic compounds. AUP with a poly(ethylene glycol) backbone molar mass of 20 kg/mol (AUP20k) was combined with three cross-linkers (pentaerythritol tetraacrylate, ethylene glycol diacrylate and AUP2k) in different double bond ratios (1:1 and 1:4). AUP20k hydrogels with the crosslinker AUP2k in a 1:1 double bond ratio revealed to have the most optimal mechanical and swelling properties. These had an absorption capacity of 32±1.6 times its own weight and a gel fraction of 80.2±4.1%. Moreover, their compressive modulus in dry and wet state was 52.4±1.0 kPa and 35.7±3.0 kPa respectively. Subsequently, the potential of Ellman's reagent and bacteria-responsive liposomes to induce a color signal upon bacterial infection was investigated. Both the Ellman's reagent and the bacteria-responsive phospholipid liposomes with the self-quenching dye 5(6)carboxyfluorescein were successfully incorporated in the hydrogels. Especially the latter showed great diagnostic potential as a fluorescent yellow signal of the hydrogel was demonstrated and quantified by colorimetry upon simulation of infection. Hydrogel wound dressings with a controlled diagnostic potential can thus early identify infections and better control the healing of burn wounds.

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Abstract **#1015**

CHITOSAN-BASED SELF-HEALING HYDROGEL FOR LIVER TISSUE ENGINEERING

Yu-Chai Tai[P][1], Yung-Te Hou[1]

[1] *Biomechatronics Engineering, National Taiwan University*

Corresponding author's email: R08631014@ntu.edu.tw

Chitosan-based hydrogels are suitable material for liver tissue engineering (LTE) owing to the similar mechanical property to native liver tissue. However, liver contains a large number of resident immune cells, which may interfere the gelation ability of highly positive-charged, chitosan-based hydrogels in vivo study. For the purpose mentioned above, we developed an injectable, chitosan-phenol (CP)-based self-healing hydrogel in this study. CP hydrogels constructed by Schiff base linkage could have responsive shape-memory ability. Besides, they could also be injected into organism without any unexpected degradation due to the in vivo influence. Primary rat hepatocytes encapsulated in CP hydrogels was carried out as an in vitro platform for evaluating cytotoxicity and hepatic functions. Our results revealed that CP hydrogels significantly enhanced the hepatocyte viability by 12.68 fold comparing to collagen hydrogels at day 5. Refer to our previous study, liver extracellular matrix (LECM) was added in CP hydrogels to create suitable microenvironment and sustain the phenotype of encapsulated hepatocytes. Interestingly, albumin synthesis of hepatocytes encapsulated in CP-LECM hydrogels was improved by 1.13 fold comparing to CP hydrogels without LECM at day 5.

In summary, CP-LECM hydrogel as a 3D culture environment can effectively enhance the viability and functions of hepatocytes. Besides, injection of self-healing hydrogels only needs simple preparation and the good biocompatibility as well as automatic reparability of self-healing hydrogel under physiological conditions also show promising potential in LTE. The dual utility of CP-LECM hydrogel may have high potential for future clinical application.

Keywords

Self-healing; hydrogel; liver tissue engineering

Abstract **#1018**

NOVEL MICROFLUIDIC PROTEIN PATTERNING TECHNIQUE FOR ORGANS-ON-CHIP APPLIED TO THE FABRICATION OF A FUNCTIONAL LIVER MODEL

Erika Ferrari[1], Giovanni Stefano Ugolini[1], Marco Rasponi[1]
[1] *Department of Electronics, Information and Bioengineering. Politecnico di Milano. Via Golgi 39, 20133, Italy*
Corresponding author's email: erika1.ferrari@polimi.it

It is well known that controlling cell-cell interactions and localization in in vitro models is paramount to better recapitulate patho-physiological conditions of human organs [1]. For instance, liver models based on micropatterned co-cultures (MPCCs) can maintain higher hepatic functions for several days in vitro compared to standard culture techniques [2]. Despite their micrometer resolution, so far MPCCs have been used in standard multi-well plates or glass substrates only. Indeed, protein patterns have never been integrated in permanently bonded microfluidic systems, thus limiting their application in miniaturized organs-on-chip models. Here we developed a novel technique that allows culture and analysis of MPCCs within a microdevice specifically designed for liver-tumor compartmentalized cultures. HepG2 cells and 3T3 fibroblast were seeded the liver compartment of the platform to generate MPCCs and HCT-116 colon cancer cells were seeded in the tumor compartment. The device was validated for pharmacokinetic-based drug screenings involving diffusion of the anticancer drug Tegafur (UFT, 100 µM) after liver metabolism (UFTL) and LIVE/DEAD observations at 24, 48 and 72h validated the hypothesis that UFT was converted into the toxic metabolite 5-Fluorouracil (5FU) by MPCCs in the liver compartment, exerting then the toxic effect on HCT-116 cells.
Acknowledgments: This work was partially financed by the Italian Ministry of Health (GR-2016-02364990 and Project MINSAL3R2016).

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Abstract **#1019**

A MECHANICALLY ACTIVE 3D GUT-ON-CHIP TO STUDY INTESTINE-MICROBIOME-TUMOR INTERACTIONS

Mattia Ballerini[P][1,2], Carlotta Catozzi[2], Paola Occhetta[1], Luigi Nezi[2], Marco Rasponi[1]

[1] *MiMic Lab, Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milano, Italy*

[2] *Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy*

Corresponding author's email: mattia.ballerini@polimi.it

It is now evident that the gastrointestinal bacteria contribute in shaping the immune system in both physiological and pathological conditions [1]. Traditional in vitro and in vivo co-culture models suffer from intrinsic limitations [2-3]. 3D-microfluidic culture systems may overcome these limitations, because they recreate complex multicellular architectures in a finely controlled dynamic environment [3-4].

In early gut-on-chip devices, relying on 2D cellular monolayers seeded on thin porous PDMS membranes, peristalsis-like mechanical actuation demonstrated to play a crucial role in model maturation [3]. More recently, the recapitulation of a 3D microenvironment proved increased faithfulness to the in vivo condition in terms of shape, functionality, and polarity [5].

Here we report a novel gut-on-chip device combining for the first time a 3D architecture with a controlled mechanical actuation. The device contains 3 independent culture units. Each unit is composed by two chambers: a cell culture compartment, containing epithelial and vascular layers separated by ECM gel, and an actuation chamber. Once the pressure in the actuation chamber increases, a controlled uniaxial strain (10%, 0.15Hz) is transferred to the epithelium layer, enhancing its maturation. Complex microbiota communities collected from healthy and pathological tissues were introduced in the gut-on-chips to evaluate the influence of different bacterial consortia on intestinal epithelium integrity.

In parallel, we developed a tumor-on-chip device consisting of patient-derived tumor spheroids embedded in ECM gel and different therapies were tested on-chip. The two modules will be integrated to study the role of gut microbiota in shaping immune system and anti-tumor therapy.

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Abstract **#1020**

IMMUNOMODULATORY BIOMATERIALS-BASED STRATEGIES TO IMPROVE SURVIVAL OF ALLOGENEIC STEM CELLS FOR CARDIAC REGENERATION

Sanjiv Dhingra[P][1], Alireza Rafieerad[1], Weiang Yan[1], Niketa Sareen[1], Keshav Narayan Alagarsamy[1]
[1] *Institute of Cardiovascular Sciences, St. Boniface Hospital Research Centre, Regenerative Medicine Program, Department of Physiology and Pathophysiology, Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada*
Corresponding author's email: sdhingra@sbrc.ca

Allogeneic (unrelated donor) stem cells including bone marrow derived mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSC) have emerged as a novel therapy for cardiac regeneration. The outcome of initial allogeneic stem cells based clinical trials was encouraging. There were no major side effects observed after transplantation of cells and implanted cells were able to improve cardiac function. However, the overall enthusiasm of lately has come down due to failure of long-term survival of transplanted cells in the recipient heart. We have performed extensive investigations to understand the mechanisms of poor survival of transplanted stem cells in the heart and demonstrated that after transplantation in the ischemic heart stem cells turn immunogenic and are rejected by host immune system. In our ongoing studies, we are interested in developing strategies to prevent immune-rejection and improve survival of transplanted stem cells in the heart. We synthesized and characterized 0D titanium carbide (Ti3C2) MXene quantum dots (MQDs). We found that MQDs possess intrinsic immunomodulatory properties and selectively reduce activation of human CD4+IFN-γ+ T-lymphocytes while promoting expansion of immunosuppressive CD4+CD25+FoxP3+ regulatory T-cells in a stimulated lymphocyte population. Furthermore, MQDs are biocompatible with MSCs and iPSC. Next, MQDs were incorporated into a chitosan-based hydrogel to create a 3D platform for stem cell delivery to the heart. This composite immunomodulatory hydrogel-based platform improved survival of stem cells and mitigated allo-immune responses. These findings suggest that this new class of biomaterials may bridge the translational gap in biomaterial and stem cell-based therapies for cardiac regeneration.

Abstract **#1022**

A NOVEL AUTOLOGOUS BONE GRAFT SUBSTITUTE CONTAINING RHBMP6 IN ABC WITH BIOCERAMICS AS A THERAPEUTIC SOLUTION FOR ACHIEVING POSTEROLATERAL SPINAL FUSION

Nikola Štoković[P][1,2], Natalia Ivanjko[1,2], Igor Erjavec[1,2], Marko Pećin[3], Sven Karlović[4], Ana Smajlović[3], Hrvoje Capak[3], Jadranka Bubić Špoljar[1], Dražen Vnuk[3], Dražen Matičić[3], Hermann Oppermann[5], Kuber T Sampath[6], Slobodan Vukičević[1,2]

[1] *Laboratory for Mineralized Tissues, School of Medicine, University of Zagreb, Zagreb, Croatia*

[2] *Scientific Center of Excellence for Reproductive and Regenerative Medicine*

[3] *Clinics for Surgery, Orthopedics and Ophthalmology, School of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[4] *Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*

[5] *Genera Research, Kalinovica, Sveta Nedelja, Croatia*

[6] *PerForm Biologics Inc, Holliston, Massachusetts, USA*

Corresponding author's email: slobodan.vukicevic@mef.hr

Posterolateral spinal fusion (PLF) is a therapeutic approach used for treatment of various orthopedic indication including degenerative disc disease, spondylolisthesis, spinal instability and symptomatic scoliosis. In this study we evaluated usage of a novel autologous bone graft substitute (ABGS) containing rhBMP6 (in two doses, 125 and 250 µg) in autologous blood coagulum (ABC) with synthetic ceramic particles (TCP or BCP) as compression resistant matrix (CRM). ABGS implants (4 per group) were implanted bilaterally between transverse processes of the lumbar vertebrae (L5-L6) following exposition and decortication in 8 New Zealand White Rabbits. Outcome of the spinal fusion was analysed after 14 weeks by microCT analyses, biomechanical testing (three-point bending test) and histologically. Successful spinal fusion was achieved in all samples. MicroCT analyses revealed that neither chemical composition of particles nor BMP dose did not affect the amount of newly formed bone. However, amount of unresorbed CRM was significantly higher in groups containing biphasic ceramics than pure TCP. Histological analysis revealed that successful osseointegration of newly formed bone with adjacent transverse processes was achieved in all specimens. Moreover, we have found that fused bone had superior biomechanical properties compared to properties of ABGS containing the same ceramics as CRM on week 7 and allograft on week 14. In this study the use of a novel ABGS formulations in a rabbit PLF model promoted the spinal fusion in rabbits and should be furher explored on larger animals.

Keywords

Bone morphogenetic proteins; Spinal fusion; Autologous blood coagulum

Abstract **#1023**

THE RGD-DWIVA PEPTIDIC PLATFORM: FROM FUNDAMENTAL STUDIES TO IN VIVO APPLICATIONS

Lluís Oliver-Cervelló[1,2], Helena Martin-Gómez[1,2], Fatima Noureddine[3], Elisabetta Ada Cavalcanti-Adam[3], Maria-Pau Ginebra[1,2,4], Carlos Mas-Moruno[1,2]

[1] Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Department of Materials Science and Engineering, Universitat Politècnica de Catalunya (UPC), 08019 Barcelona, Spain

[2] Barcelona Research Center in Multiscale Science and Engineering, UPC, 08019 Barcelona, Spain

[3] Department of Cellular Biophysics, Max Planck Institute for Medical Research, 69120 Heidelberg, Germany

[4] Institute for Bioengineering of Catalonia (IBEC), 08028 Barcelona, Spain

Corresponding author's email: lluis.oliver@upc.edu

Recreating the healing microenvironment of bone tissue is of paramount importance to ensure the success of implantable materials. In this regard, surface biofunctionalization with integrin-binding molecules combined with growth factor derived sequences stands out as a promising approach [1-2].

Herein, the synthesis of a multifunctional peptidic platform, composed of the cell adhesive RGD peptide and the DWIVA sequence, derived from the wrist epitope of BMP-2 [3], and its application to diverse biomaterials, is presented.

Preliminary experiments with C2C12 myoblasts on glass model substrates demonstrated the ability of the biomimetic interface to enhance cell adhesion, their transdifferentiation capacity to the osteoblastic lineage and BMP-2 dependent signaling through a Smad-independent pathway [4]. These results were further supported by human mesenchymal stem cells (hMSCs) studies, in which cell adhesion and mineralization were enhanced in comparison to the controls [4]. Interestingly, the translation of this strategy to clinically relevant biomaterials, such as titanium and hydrogels, was successfully performed. In detail, the MSC behavior on both biomaterials was assessed by means of cell adhesion, proliferation and osteodifferentiation (ALP activity, mineralization and gene expression) [5]. Due to satisfactory results, in vivo experiments were also done: coated titanium screws were implanted on rat calvaria, to assess osteointegration, while the functionalized hydrogels were used to evaluate stem cell mineralization and bone formation on a non-osseous ectopic environment in mouse [5].

In summary, our experimental data proved the feasibility of our strategy and highlights the potential of biomimetic peptides on bone tissue engineering.

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Abstract **#1027**

MARKER-INDEPENDENT ANALYSIS OF HUMAN MACROPHAGE SUBSETS BY NON-INVASIVE RAMAN SPECTROSCOPY

Nora Feuerer$_P$[1,2], Julia Marzi[1,2,3], Eva Brauchle[1,2,3], Daniel A. Carvajal Berrio[2,3], Christopher Shipp[1], Katja Schenke-Layland[1,2,4]

[1] The Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany

[2] Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany

[3] Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University Tübingen, Germany

[4] Dept. of Medicine/Cardiology, University of California Los Angeles (UCLA), Los Angeles/CA, USA

Corresponding author's email: nora.feuerer@nmi.de

The main contributors of the immunological response towards an implant are monocytes and monocyte-derived macrophages (MDM), cells that are highly sensitive to material features such as surface structure, chemistry and wettability.

By adopting different polarization states, such as inflammatory (M1), regenerative (M2a) or fibrotic (M2c), MDMs can substantially influence the healing process around an implant.

However, existing methods that can comprehensively analyze the molecular response of these cells towards an implant are invasive and in most cases marker-based. Aggressive treatments to detach adherent cells such as scraping or digestion with enzymes alter the cell surface proteins, impair cell viability and ultimately limit the ability to accurately characterize adherent cells.

Raman spectroscopy is a marker-independent, non-invasive imaging method that allows the analysis of either fixed or living cells without the need for staining or processing. It is a powerful tool to localize and characterize biochemical components such as proteins, lipids and nucleic acids in single cells, making it an ideal method to reveal differences in the chemical composition of MDM subsets.

In this work, in vitro cultured human MDMs were polarized towards M1, M2a and M2c and analyzed by Raman spectroscopy.

Our findings reveal major differences across donors between MDM subtypes and provide new insights into physiological differences between macrophage polarization states. In addition, we could show that Raman spectroscopy can be employed on MDMs adherent to common implant materials to further characterize MDM-material interaction in vitro.

Abstract **#1028**

LONG-TERM MECHANICAL LOADING IS REQUIRED FOR THE FORMATION OF 3D BIOPRINTED FUNCTIONAL OSTEOCYTE BONE ORGANOIDS

Jianhua Zhang$_P$[1], Marsel Ganeyev[1], Anna-Katharina Zehnder[1], Anke de Leeuw[1], Gian Nutal Schädli[1], Julia Griesbach[1], Marina Rubert[1], Ralph Müller[1]

[1] *Institute for Biomechanics, ETH Zürich, Zürich, Switzerland*

Corresponding author's email: jianhua.zhang@hest.ethz.ch

Organoids are 3D in vitro culture systems derived from self-organizing stem cells, which have been rapidly applied to understanding stem cell biology, organogenesis, and various human pathologies. However, developing an in vitro 3D osteogenic organoid that supports osteoblast-osteocyte differentiation and mimicks the in vivo conditions has not been achieved yet. Here, 3D bioprinted human mesenchymal stem cells (hMSC)-laden graphene oxide composite scaffolds were cultured in cyclic-loading bioreactors for up to 56 days to investigate the influence of mechanical loading on development of an osteogenic organoid. Our results showed that mechanical loading significantly enhanced collagen I maturation with a higher percentage of matured collagen I, fiber width and length. Mechanical loading from day 1 (ML01) significantly increased early cell proliferation, cell spreading morphology (day 31), scaffold mineral density (day 49 and 56), stiffness (day 56), as well as osteocalcin gene and protein expression (day 56) compared to non-loading (NL) and mechanical loading from day 21 (ML21). Importantly, ML01 stimulated osteocyte differentiation with significantly more osteocyte-related genes (DMP1 and SOST) and higher osteocyte-related proteins (PDPN and SOST) expression at day 56 than NL and ML21. Meanwhile, mechanical loading up-regulated YAP expression in the cytoplasm and extracellular matrix. Our results demonstrate that mechanical loading and loading initiation time play a critical role in the regulation of self-organizing osteogenic organoid formation exhibiting high mineralization and a co-culture of differentiated osteoblasts and osteocytes. Such 3D osteogenic organoids may serve as a human-specific alternative to animal testing for the study of bone pathophysiology and drug screening.

Abstract **#1030**

DEVELOPMENT OF ELECTROCONDUCTIVE BIOHYBRID SCAFFOLDS FOR IN VITRO CARDIAC MODELS

Matteo Solazzo[P][1,2], Michael G. Monaghan[1,2,3]

[1] *Trinity Centre for Biomedical Engineering, Trinity College Dublin, Dublin, Ireland*

[2] *Department of Mechanical, Manufacturing and Biomedical Engineering, Trinity College Dublin, Dublin, Ireland*

[3] *AMBER Centre, Dublin, Ireland*

Corresponding author's email: solazzom@tcd.ie

Despite advances, myocardial infarction (MI) remains a leading cause of death in western countries [1]. Current treatments act to curb symptoms and progression of the eminent scar tissue but a true tissue engineering platform is challenging due to the low regeneration potential of the myocardium. Electroconductive biomaterial scaffolds and electrical stimulation can improve the differentiation of progenitor stem cell sources into cardiomyocytes and the maturation of cardiac engineered organoids that could realise more effective therapies [2].

In this study, poly (3,4-ethylenedioxy thiophene):polystyrenesulfonate (PEDOT:PSS) was functionalized with a novel combination of stabilizing molecules and processed into three-dimensional scaffolds using freeze-drying. The optimization of different manufacturing parameters resulted in highly porous constructs with tailored dimensions and aligned or isotropic micro-architecture that could be created repeatably with ease. In particular, three-dimensional electroconductive scaffolds showed biomimetic anisotropy typical of the human myocardium, in terms of mechanical and electrical features within ranges that meet that of the myocardium to induce myogenic maturation [3, 4]. These platforms were seeded with cardiomyocytes and incorporated into a custom-made electrical pacing bioreactor for investigation of the effects of scaffold features and field direction on cells orientation and tissue maturation via fluorescent imaging and quantitative assays . Finally, the combination of these electroconductive substrates with a biologically derived component generated a biohybrid platform able to perform as an optimal substrate for cardiac tissue engineering applications.

Keywords

Three-dimensional electroconductive scaffolds; Cardioinductive matrix; PEDOT:PSS

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Abstract **#1032**

MESENCHYMAL STEM/STROMAL CELLS IN CLINICAL TRIALS: CRITICAL ANALYSIS OF BIOLOGICAL CHARACTERISATION AND ITS IMPLICATIONS FOR REGENERATIVE MEDICINE

Alison Wilson[P][1], Emma Rand[1], Andrew Webster[1], Paul Genever[1]

[1] *University Of York*

Corresponding author's email: ajw638@york.ac.uk

We analysed the extent of characterisation data reported in mesenchymal stem/stromal cell (MSC) clinical trials. In this widely used and heterogeneous cell type, the extent of data defining identity, purity and functionality of the investigational medicinal product (IMP) is extremely variable.

Our dataset of 84 clinical studies covered reports from 27 countries across 35 indications. 64% of trials were Phase 1, 33% Phase II and 2% Phase III. MSC characterisation was absent in 32 studies (38.1%). The majority (41 studies, 48.8%) reported average values per cell surface marker for all cell lots used in the trial, with 11 (13.1%) studies reporting actual values for each cell lot. 48 studies (57%) discussed cell viability. Although multi-potency is a key characteristic of MSCs, only 29% discussed osteogenesis, with chondrogenesis being assessed in 20% of studies. Potency assays were addressed in 7 papers. Extent of characterisation was not related to clinical phase of development.

A subset of markers recommended by the International Society Cell and Gene Therapy Society (ISCT)[1] for identification of MSCs formed the most frequently reported characteristic: CD45 in 56 studies, CD105 (51 studies), CD90 (49 studies), CD34 and CD73 (48 studies). Overall, only 62% of the studies reported critical attributes of the IMP.

Characterisation of the IMP was poorly established in this dataset. The term “MSC” is insufficient to define key attributes including identity, purity and biological activity: we strongly recommend that clinical trial papers report a minimal level of consistent characterisation data to enable assessment of results in context.

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Abstract **#1033**

ENGINEERING AN EX-VIVO PERFUSION-BASED BIOREACTOR SYSTEM FOR DRUG-TESTING ON PATIENT-SPECIFIC CANCER SPECIMENS

Manuele Giuseppe Muraro[P][1], Andrea Mazzoleni[1], Evrim Ceren Kabak[1], Arnaud Scherberich[1], Ivan Martin[1]
[1] *Tissue Engineering, Department of Biomedicine, University of Basel and University Hospital of Basel*
Corresponding author's email: manuele.muraro@usb.ch

INTRODUCTION: Close mimicking of pathophysiological features of diseased tissue is key for drug discovery and precision medicine. Ex-vivo models represent a promising possibility to assess patient-specific drug response, allowing the preservation of cancer, stromal and immune cells, and tissue extracellular matrix. Here, we developed perfusion bioreactor-based engineered models able to culture patient-derived cancer tissues and to test the sensitivity to chemotherapies in use for breast and colorectal cancer.

METHODS: Freshly excised tissue specimens fragments were inserted between two collagen type-I sponges and cultured for up to 10 days in a perfused-based system. Static and perfused cultures are compared on proliferation (Ki67), apoptosis (cC3), and maintenance of the cellular components (EpCAM, Vim, CD45) via histomorphological analysis. In a second step, hormonal (Fulvestrant), or anti-proliferative (5FU) treatment was tested.

RESULTS: We observed cellular and architectural cancer tissue maintenance over 14 and 5 days with breast1 and colorectal2, respectively, allowing clinically relevant tissue responses and the study of the interactions between stromal, immune and cancer cells under different treatment regimes.

CONCLUSION & FUTURE PERSPECTIVE: In the era of personalized medicine, ex-vivo culture of cancer tissue represents a promising approach to generate clinically relevant responses to drugs or immunotherapy strategies, which 2D cultures cannot mimic. We will further develop the bioreactor to investigate the influence of bone microenvironment on metastatic process and to be fit for smart throughput drug-testing on patient-derived ex-vivo tumor samples.

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Abstract **#1034**

TRILINEAGE POTENCY OF HUMAN NUCLEUS PULPOSUS CELLS BEFORE AND AFTER CRYOPRESERVATION

Andreas Croft$_P$[1]

[1] *Tissue Engineering for Orthopaedics & Mechanobiology (TOM), Department for BioMedical Research (DBMR) of the Medical Faculty of the University of Bern, University of Bern, Bern, Switzerland.*

Corresponding author's email: andreas.croft@dbmr.unibe.ch

Recently, various progenitor cells have been found within the nucleus pulposus (NP) of human intervertebral discs (IVD). However, little is known about the effect of expansion and cryopreservation on here called “heterogenic” human NP cells (hNPCs) and their stemness. Therefore, we aimed to expand hNPCs while investigating their differentiation potential before and after cryopreservation and to find an optimal approach to cryo-preserve them.

HNPCs were obtained from six patients undergoing spinal surgery. Cells were then differentiated into osteogenic, adipogenic or chondrogenic lineages for 21 days or were cryo-preserved for one week at -150°C with five different cryopreservation media to compare their effect on the cell’s viability and differentiation potential. Cell viability was determined by cytometry using propidium iodide. The differentiation potential was assessed using qPCR and histology.

HNPCs showed the presence of lipid droplets and upregulated adiponectin (up to 3,000-fold). Furthermore, cells cultured in chondrogenic medium expressed up to 1,000-fold more collagen type 2 and released up to 13% more glycosaminoglycans into the supernatant than controls. However, osteogenic differentiation was less pronounced and was subjected to large donor variability. Nevertheless, calcium deposits were occasionally visible. After cryopreservation, the hNPCs’ cell viability was comparable for all five tested cryopreservation media (~82% cell viability) and cryopreservation did not significantly affect the cell’s stemness.

HNPCs can differentiate into a chondrogenic lineage and more unexpectedly, also into an adipogenic and partially osteogenic lineage. Furthermore, all five tested cryopreservation media seem to perform the same in terms of cell viability and maintaining hNPCs' stemness.

Abstract **#1035**

ELECTROSPUN 3D OPEN-NETWORK SCAFFOLDS SUPPORT PENETRATION AND VIABILITY OF TRABECULAR MESHWORK CELLS

Devon Crouch[P][1], Carl Sheridan[1], Raechelle D'Sa[2], Lucy Bosworth[1]

[1] *Department of Eye and Vision Science, Institute of Life Courses and Medical Sciences, University of Liverpool*

[2] *School of Engineering, University of Liverpool*

Corresponding author's email: d.crouch@liverpool.ac.uk

In 2020, an estimated 76 million people will be diagnosed with glaucoma, which remains the second leading cause of irreversible blindness worldwide1. The trabecular meshwork (TM) - a highly porous tissue located in the anterior chamber of the eye - drains fluid from the eye, which thus maintains intraocular pressure. However, the TM can become blocked, which raises this pressure and causes compression of the optic nerve, ultimately leading to sight loss.
Electrospun scaffolds closely mimic the porous 3D architecture of TM tissue and can be further tailored to influence depth of cell penetration and material properties, such as stiffness. Conventional electrospinning was compared to cryo-electrospinning (mandrel collector containing dry ice). Poly(ε-caprolactone) yielded fibres of comparable diameter (0.64±0.14 µm conventional vs. 0.56±12 µm cryo) but significant changes to the tensile stiffness were observed (4.12±1.50 MPa conventional vs. 0.79±0.41 MPa cryo). Yet, cryo-scaffolds remained handle-able. Human TM cells adhered and proliferated on both scaffolds but stayed localised to the outer fibre surface for conventional scaffolds, whereas cells penetrated to 54% of the total depth for cryo-scaffolds after 7 days in culture. Further studies will continue to optimise a scaffold that is supportive of TM cell function that may be used in a reproducible in vitro model and as a potential tissue-engineered device to restore and regenerate the TM, and hence prevent the onset of glaucoma.

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Abstract **#1036**

VISCOSITY MATTERS: VISCOELASTIC HYDROGELS DRIVE CHONDROGENESIS OF MESENCHYMAL STEM CELLS

Matthew Walker$_P$[1], Marco Cantini[1]

[1] *Centre for the Cellular Microenvironment, University of Glasgow, UK*

Corresponding author's email: matthew.walker@glasgow.ac.uk

While the stiffness of elastic hydrogels has been long recognized to determine human mesenchymal stem cell (hMSC) differentiation, viscous interactions are only recently emerging as powerful regulators of stem cell fate (1). Indeed, viscoelastic hydrogels, where mechanical stresses relax over time, have been shown to promote hMSC spreading and proliferation, as well as formation of an interconnected bone matrix from osteoblasts. On the other hand, elastic hydrogels can inhibit cell proliferation and, in the case of chondrocytes, limit cartilage matrix formation (2). Hydrogels can also be functionalized with peptide motifs to improve hMSC attachment, as well as influence cell fate: for example, chondrogenesis can be controlled by peptide gradients using integrin receptor RGD and cadherin ligand HAVDI (3). However, investigations into how the viscoelastic properties of hydrogels influence the chondrogenic differentiation of hMSCs are lacking. In this work, we investigate whether viscoelastic hydrogels, functionalized with specific peptide combinations, can provide a cellular microenvironment conducive to the chondrogenic differentiation of hMSCs. We designed RGD-functionalized polyacrylamide hydrogels with variable viscous properties and consistent elasticity and we found that an increase in viscosity promoted the expression of both early and late chondrogenesis markers, while preventing chondrocyte hypertrophy, via the modulation of mechanosensitive pathways (adhesion proteins, YAP and nuclear lamins). Promotion of cadherin ligation via HAVDI/RGD functionalization of the gels further affected mechanotransduction and promoted chondrogenesis. Finally, translation of these optimal chondrogenic gel properties to biodegradable polyethylene glycol hydrogels demonstrated that increased substrate viscosity promotes enhanced 3D chondrogenesis by hMSCs.

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Abstract **#1037**

MECHANICAL MODELING OF BIOMIMETICALLY TEXTILE REINFORCED SOFT TISSUE FOR BIOHYBRID HEART VALVES

Mahmoud Sesa$_P$[1], Lukas Lamm[1], Christian Böhm[2], Stefan Jockenhövel[2], Stefanie Reese[1], Hagen Holthusen[1]

[1] *RWTH Aachen University, Institute of Applied Mechanics*

[2] *RWTH Aachen University, Institute of Applied Medical Engineering*

Corresponding author's email: mahmoud.sesa@ifam.rwth-aachen.de

The long-term function of biohybrid implants (e.g. heart valves) is significantly related to the production of the extracellular matrix (ECM) with regard to the quantity and quality. The integration of a load oriented textile structure into the implant serves as (i) a biomimetic reinforcement and (ii) a guide for the direction of growth of the ECM. There is an urgent demand for suitable models to simulate the implant under in vitro and in vivo conditions and to further support the design planning phase.In the presentation, we will focus at first on the constitutive modeling of growth and remodeling of ECM. The densities of collagen and elastin are treated as internal variables. Additionally, anisotropic growth is considered by means of a multiplicative decomposition of the deformation gradient into an elastic and a growth part. The textile reinforcement causes macroscopically anisotropic behaviour which is taken into account by means of structural tensors. The entire constitutive framework is embedded into a special finite element technology which enables the efficient computation of thin structures like heart valves. The contribution ends with a finite element simulation of an idealized heart valve. The results are compared with experimental data available at BioTex (Institute of Applied Medical Engineering, RWTH Aachen University, Germany).

Abstract **#1038**

PIVOTAL IMPORTANCE OF REINFORCEMENT OF CARTILAGE IMPLANTS CONFIRMED IN CHALLENGING LARGE ANIMAL MODEL; PRESENCE OF TRANSPLANTED CELLS PROBABLY SECONDARY

Mylene de Ruijter$_P$[1], Paweena Diloksumpan[2], Inge Dokter[1], Harold Brommer[2], Ineke H. Smit[2], Riccardo Levato[1,2], René van Weeren[2], Jos Malda[1,2], Miguel Castilho[1,3]

[1] *Department of Orthopaedics, RMCU Utrecht, UMC Utrecht, University of Utrecht, The Netherlands*

[2] *Epartment of Clinical Sciences, faculty of Veterinary Medicine, Utrecht University, The Netherlands*

[3] *Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands*

Corresponding author's email: m.deruijter@umcutrecht.nl

In articular cartilage, the collagen arcades provide the tissue with its extraordinary mechanical properties. As these structures cannot be restored once damaged, functional restoration of articular cartilage defects remains a major challenge. We report that the use of a reinforced osteochondral implant, based on a gelatin methacryloyl cartilage phase, reinforced with precisely patterned melt electrowritten polycaprolactone micrometer-scale fibres in a zonal, cartilage-mimicking fashion, can provide long-term mechanically stable neo-tissue in an orthotopic large animal model. Interestingly, the cell-free implants, used as a control in this study, showed abundant cell ingrowth and similar favourable results as the cell-containing implants. Our findings underscore the hypothesis that mechanical stability is more determining for the success of the implant than the presence of cells and pre-cultured extracellular matrix. This observation is of great translational importance and highlights the aptness of advanced 3D (bio)fabrication technologies for functional tissue restoration in the harsh articular joint mechanical environment.

Abstract **#1039**

SCALING UP FROM OSTEOCHONDRAL PLUG TO PATIENT-SPECIFIC CONDYLE RESURFACING: FABRICATION, IN VITRO CHARACTERIZATION, AND MECHANICAL CHARACTERIZATION UNDER PHYSIOLOGICAL CONDITIONS OF CLINICALLY RELEVANT OSTEOCHONDRAL IMPLANTS

Mylene de Ruijter$_P$[1], Nasim Golafshan[1], Inge Dokter[1], Ioanna Gkoni[1], Roderick Verberne[2], René van Weeren[3], Pieter A.J. Brama[4], Daniel J. Kelly[5,6,7,8], Keita Ito[1,9], Paige Little[2], Peter Pivonka[2], Jos Malda[10,3], Miguel Castilho[10,9]

[1] *Department of Orthopaedics, Regenerative Medicine Utrecht, UMC Utrecht, University of Utrecht, The Netherlands.*

[2] *Science and Engineering Faculty, School of Mechanical Medical & Process Engineering, Queensland University of Technology, Brisbane, Australia*

[3] *Department of Clinical Sciences, Utrecht University, The Netherlands*

[4] *School of Veterinary Medicine, University College Dublin, Dublin, Ireland*

[5] *Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland*

[6] *Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Ireland*

[7] *Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland*

[8] *Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland*

[9] *Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands*

[10] *Department of Orthopaedics, Regenerative Medicine Utrecht, UMC Utrecht, University of Utrecht, The Netherlands*

Corresponding author's email: m.deruijter@umcutrecht.nl

Articular cartilage defects are common and current surgical treatments provide sub-optimal tissue repair. Multiple biofabrication approaches have tried to recreate the chondral architecture in biochemical and structural composition and this has frequently been combined with an osteal anchor in the form of an osteochondral plug. In this study, the translation from this relatively small plug to larger clinically-relevant and patient-specific implants is explored. Osteochondral patient-specific large (surface area = 469 mm2) implants consisted of a porous bone component, close cartilage-to-bone interface, and a microfibre reinforced cell-laden gelatin methacryloyl (gelMA) cartilage component. The effect of implant size (bone component only) on stress-strain distribution and cartilage-like tissue formation is studied by means of axial compression and compression under 50° and 60° flexion angles using a robotic arm system. The implant stiffness decreased with increased implant size. An increase in size of the cartilage component (from 6 mm diameter discs to 24 mm diameter discs) did not hamper cartilage-like tissue formation in vitro. As the larger implants include a patient-specific complex shaped geometry, a software tool has been developed and validated to automatically generate a numerical control programming language (i.e., g-code) that resurfaces a bone structure with melt electrowritten (MEW) microfibres based on a 3D-standard tessellation language (.STL) file. As a proof of principle, a large complex shaped implant was fabricated and cultured in vitro to assess 3D matrix distribution. This study shows the first steps in translating from osteochondral plugs to larger patient-specific implants.

Keywords

Bioprinting; Melt electrowriting; Large osteochondral implants

Abstract **#1040**

CELL BEHAVIOR ON CURVED 2.5D SUBSTRATES COMBINED WITH EXTRACELLULAR MATRIX PROTEIN PATTERNS

Cas van der Putten[1,2], Antonetta Buskermolen[1,2], Maike Werner[1,2], Hannah Brouwer[1,2], Thomas Woud[1,2], Carlijn Bouten[1,2], Nicholas Kurniawan[1,2]

[1] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands*

[2] *Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands*

Corresponding author's email: c.v.d.putten@tue.nl

Various structural cues present in the extracellular matrix (ECM), e.g. topography and ligand distribution, have been shown to influence cell behavior and function [1]. Until now, these structural cues are studied using single-cue platforms that lack the complexity of the multi-cue ECM. In order to overcome this limitation, we developed an approach that combines optics-based protein patterning (resembling contact-guidance cues) and lithography-based microfabrication (resembling topographical cues) enabling the high-throughput investigation of multi-cue cellular environments.

We first explored the versatility of the approach in terms of chip material (PDMS, polystyrene) in combination with ECM proteins for patterning (fibronectin, collagen type I and IV, gelatin). In order to investigate cellular responses to the multi-cue substrates, human bone marrow stromal cells and vascular-derived human myofibroblasts were cultured on the physiologically relevant multi-cue environments and cell orientation was quantified. On concave, patterned structures ($1/2500 \leq \kappa \leq 1/125$ µm-1), human myofibroblasts predominantly oriented in the direction of the contact-guidance pattern. In contrast, for myofibroblasts and human bone marrow stromal cells on micropatterned convex substrates with curvatures of $\kappa \geq 1/1000$ µm-1 and $\kappa \geq 1/500$ µm-1 respectively, the majority of cells aligned into the longitudinal direction of the 2.5D features, indicating that cells followed the structural cues from the curvature instead.

These findings reveal non-trivial and non-additive effects of multiple environmental cues on cell adhesion and phenotype. Drawing comparisons from the responses of other cell types (endothelial cells/corneal keratocytes) for which tissue curvature is equally crucial, we will discuss the relevance of this approach for understanding how multiple cues shape cellular response.

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Abstract **#1041**

DEVELOPMENT AND CHARACTERISATION OF SELF-ASSEMBLING PEPTIDE HYDROGELS AS BIOINKS FOR 3D BIOPRINTING APPLICATIONS

Albert Ginjume$_P$[1,2], Alberto Saiani[1,2], Judith Hoyland[3], Cosimo Ligoro[1,2]

[1] *Manchester Institute of Biotechnology (MIB), The University of Manchester, Oxford Road, Manchester, M13 9PL, UK*

[2] *School of Materials, Faculty of Science and Engineering, The University of Manchester, Oxford Road, Manchester, M13 9PL, UK*

[3] *Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Oxford Road, Manchester, M13 9PL, UK*

Corresponding author's email: albert.ginjaume@postgrad.manchester.ac.uk

With a growing demand for highly effective therapies, more sophisticated tissue-engineered models are required for a better understanding of the fundamental biological processes that underlie regeneration. The state-of-the-art technology of 3D bioprinting has the scope to achieve well-defined biological structures by printing cell-embedded hydrogels or bioinks. One of the main challenges in obtaining fully functional constructs is the lack of optimal bioinks, which not only require good fabrication properties but also a cell-friendly extracellular matrix (ECM)-like microenvironment. Self-assembling peptide hydrogels (SAPHs) are fully defined and nature occurring hydrogels with tuneable mechanical properties. As a result, SAPHs stand as a powerful alternative to existing hydrogels in biofabrication.

Due to the lack of suitable biomaterials for 3D bioprinting applications, this research aims to design SAPHs as advanced bioinks for extrusion-based 3D bioprinting. Printability was assessed for the Manchester BIOGEL family of SAPHs, PeptiGel® hydrogels. Rheological analyses showed that peptide-based hydrogels are shear thinning and recover well under shear stress. Relaxation times fitting revealed the characteristic dynamic times in which our hydrogels recovered following the classical mechanical model. Printability experiments showed PeptiGels® are favourable in enhancing high-resolution constructs and therefore, comparable to materials with good fabrication properties. Furthermore, PeptiGels® were modified with sodium chloride, which tuned the ionic strength and therefore the hydrophobicity of the fibres within the hydrogel increasing its mechanical strength.

Thus far, we have successfully developed and tested peptide-based biomaterial inks. Current work aims to investigate their biocompatibility and potential as prospective cell-embedded bioinks for bone and cartilage fabrication.

Keywords

Self-assembling peptide hydrogel; Bioinks; Printability

Abstract **#1043**

RECONSTRUCTION OF CRANIAL VAULT DEFECTS USING PATIENT SPECIFIC IMPLANTS.

Thomas Zegers$_P$[1], David Koper[1,2,3], Mariel Ter Laak-Poort[4], Peter Kessler[5]

[1] *Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Center, Maastricht.*

[2] *MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands*

[3] *GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, the Netherlands*

[4] *Department of Neurosurgery, Maastricht University Medical Center Maastricht.*

[5] *Department of Cranio-Maxillofacial Surgery,Maastricht University Medical Center Maastricht.*

Corresponding author's email: thomas.zegers@mumc.nl

Objective:

Large skull bone defects of the cranial vault can result from various reasons. Reconstruction of these defects is performed for protective and aesthetic reasons but is also required for adequate intracranial homeostasis. Autogenous bone has traditionally been used as the material of first choice. Complications like resorption or infection of the bone specimen, or just the extent or complex anatomy of the defect, frequently form the necessity of an alternative. In recent years patient-specific skull implants have become the most valuable alternative. We reviewed our own patient data to assess pre- and intraoperative aspects, complications en experiences in PSI cranioplasty

Methods:

We retrospectively evaluated all patients receiving a PSI between 2004 and 2020 at Maastricht University Medical Center. These cases were analyzed for demographics, perioperative surgical and medical aspects and costs.

Results:

37 consecutive patients receiving PSI cranioplasty were included. Twenty-five patients received PEEK implants and twelve patients received titanium implants. 28 patients have had at least one previous reconstruction. Postoperatively, two patients experienced complications (5.4%). The average costs of a PSI skull implant was €7598 euro (range € 3876 -€10846).

Conclusion:

Treatment of neurocranial defects requires an optimal preoperative planning and intraoperative procedure. The number of complications was relatively low compared to reports in literature. PSI cranioplasty can be regarded as a valuable alternative to the tradional methods of reconstruction and may be seen as the new gold standard.

Abstract **#1044**

A ROBUST MICROPHYSIOLOGICAL SYSTEM FOR MECHANISTIC INVESTIGATION OF FAT-BONE CROSSTALK IN DIABETES

Hang Lin$_P$[1], Xiurui Zhang[1], Benjamen O'Donnell[2], Bruce Bunnell[3]

[1] *University of Pittsburgh*

[2] *Tulane University*

[3] *University of North Texas Health Science Center*

Corresponding author's email: hal46@pitt.edu

The increased risk of fragility fractures (fractures that occur from minimal trauma) has recently been recognized as a significant complication of type diabetes (T2D). Given the complexity of diabetes, no single factor or pathway has been identified to govern T2D-induced bone fragility. Recently, factors produced by the adipose tissue, adipokines, have drawn more attention due to often observed fat dysfunction in T2D. In this study, our goal is to create a human micro-adipose (μAD)/micro-bone (μBO) tissue chip, which will provide a model to study the interplay between these two tissues in T2D, and also serve as a high-throughput platform for specialized drug screening. Specially, μAD and μBO tissues are housed in a customized bioreactor and microfluidically connected to each other. With this novel tissue chip, we hypothesize that the dysfunction of adipose tissues in T2D will result in diminished bone turnover. Given the importance of adiponectin on bone health, we also hypothesize that increasing adiponectin expression by pharmacologically enhancing T2D adipose health will reduce T2D-induced bone fragility. Currently, the normal μADs/μBOs tissue chip has been created from human stem cells, in which μADs and μBOs are able to maintain respective phenotype up to 4 weeks, as confirmed by real time-PCR and histology assay. Currently, we are examining the role of adiponectin on bone health through gain or loss-of-function test. Next, we will investigate the impact of T2D μADs on the health of μBOs, and test the applicability of targeting adipose tissues as a new treatment strategy for T2D-associated bone fragility.

Abstract **#1045**

INNOVATIVE BONE SUBSTITUTE CARRIER WITH INTEGRATED ANTI-CANCER PEG-MODIFIED CURCUMIN INHIBITS OSTEOSARCOMA CELL GROWTH IN VITRO

Mingjie Wang[P][1,2], Chunfeng Xu[1], Dong Xu[3], Yuxin Wan[3], Chang Du[3], Jenneke Klein-Nulend[2], Yuelian Liu[1]

[1] Department of Oral Implantology and Prosthetic Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands

[2] Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands

[3] Department of Biomedical Engineering, School of Materials Science and Engineering, South China University of Technology, Guangzhou, China

Corresponding author's email: m.wang@acta.nl

Tumor resection is incomplete in 75% of craniofacial osteosarcoma patients. The remaining osteosarcoma causes tumor-recurrence. There is an urgent need for new treatment modalities to prevent this tumor-recurrence. The aim of this study was to develop an anticancer-drug local sustained-slow-release-system. We incorporated modified-curcumin (mCur) into octacalcium phosphate (OCP), determined release kinetics of mCur from mCur-incorporated OCP (mCurOCP) granules, and tested the effect of mCurOCPgranules on MG63 human osteosarcoma cell growth.

mCur release from mCurOCP granules was assessed at pH7.4 (simulating healthy tissue) and pH6.5 (simulating tumor tissue) up to 6 weeks. Osteosarcoma cells were cultured in the presence of OCP-granules with/without mCurOCP for 48h. Cell number was assessed by light microscopy, and cell metabolic activity by MTT-assay.

A linear relationship was found between the mCur concentration in the calcium phosphate-containing solution and mCur incorporated in mCurOCP (R2=99%). mCur release was significantly higher at pH6.5 (76.4±4.9%; mean±SEM) than at pH7.4 (28.5±1.1%). Cell metabolic activity was not affected by OCP-granules. However, mCurOCP-granules (0.1mg/well) decreased cell density by 2.1-fold. mCurOCPgranules at 0.8mg/well significantly inhibited osteosarcoma cell metabolic activity (8.2±1.3% of control), while 0.05, 0.1, 0.2, and 0.4mg/well had less effect.

In conclusion, our data show that mCur was successfully incorporated in OCP, and mCur was slowly and sustained released from mCurOCP granules, while its release rate was pH-dependent. The inhibitory effect of mCurOCP granules on osteosarcoma cell growth suggests that this innovative anticancer bone-substitute is promising for local application in the surgical area to prevent tumor-recurrence.

Keywords

Osteosarcoma; Curcumin; Bone substitute carrier

Abstract **#1046**

THE EFFECTS OF TANNIC ACID ON AMELIORATING HEPATIC FUNCTIONS AND HEPATOPROTECTIVE

Yung-Te Hou$_P$[1]

[1] *National Taiwan University*

Corresponding author's email: r08631039@ntu.edu.tw

Traditional Chinese herbal medicine has been promoted as a new drug for many applications. In this research, we aimed to develop a new application in liver tissue engineering of tannic acid (TA), which is a plant polyphenol commonly used in food chemistry. TA is a nature substance in green tea, and some researches indicated that polyphenols in tea might have some hepatoprotective effect in vitro, but the mechanism of these polyphenols on liver tissue engineering are still unclear. In this study, the benefit of TA for improving hepatics functions and alleviating the hepatotoxicity was investigated in vitro for further clarifying the mechanism of polyphenol.

Results have revealed that: (1) The optimized concentration of TA for enhancing hepatic functions were around 0.5 to 5 μg/mL; (2) TA was effective for hepatocyte culture (relative viability was increased by 10% at day 3; albumin synthesis was increased by 18%from day 2 to day 3; and albumin expression was increased by 210% at day 2) and repaired injured hepatocytes from CCl4 (cytotoxicity was decreased by 40% to 90% when the hepatocytes was treated with CCl4 for 10 to 18 hr, separately).

In conclusion, this study showed that TA have the potential for enhancing the hepatic functions in a specific concentration, furthermore, the hepatocytes injured by CCl4-containing medium (for simulating the toxin-mediated liver fibrosis in vitro) could be recovered to the preinjury state after TA-containing medium treatment. TA may also have promising effects against hepatic fibrosis in vivo.

Keywords

Liver tissue engineering; Tannic acid; CCl4-induced liver injury

Abstract **#1047**

THE INTERACTION BETWEEN SUN1 AND NUP153 MAY RESULT IN THE TRANSMISSION OF EXTERNAL FORCES TO THE NUCLEAR PORE COMPLEX

Francesca Donnaloja[1], Anthony Schuller[2], Emanuela Jacchetti[1], Monica Soncini[3], Thomas U. Schwartz[2], Manuela T. Raimondi[1]

[1] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy

[2] Department of Biology, Massachusetts Institute of Technology,Cambridge, MA 02139, USA

[3] Department of Electronics, Information and Bioengineering, Politecnico di Milano,Milan, Italy;

Corresponding author's email: francesca.donnaloja@polimi.it

Stem cell fate strongly correlates to nuclear morphology. We have previously shown that stem cells cultured in a 3D artificial "nichoid" microscaffold maintain a roundish configuration, a higher level of stemness and a reduced molecular exchange with the nucleus when compared with cells spread on 2D controls [1]. We hypothesize that forces transmitted to the nucleus via integrins, actins, nesprins and SUN proteins affects the nuclear pore complex (NPC) architecture and transcription factor import/export through the nuclear envelope [2]. Here, we investigate the interaction of the nuclear envelope protein SUN1 with Nup153, protein of the nuclear portion of the NPC.

We designed several Nup153 constructs that spanned the entire protein sequence. For SUN1, we identified its nucleoplasmic domain as the region of interest for interaction with Nup153. We tested Nup153-SUN1 protein interactions using Bio-layer interferometry. We detected at least 2 different binding sites in the N-terminal region of Nup153. Neither the C-terminal nor the Zinc-finger domain of Nup153 interacts with SUN1. This result suggests that SUN1 could transmit external forces directly to the nuclear basket at Nup153. These forces may be responsible, at least in part, in a rearrangement of the nuclear basket affecting the molecular flux through the nuclear envelope shown in literature. Our next steps will be to crystallize the SUN1-Nup153 complex to solve its structure and to implement a molecular dynamics simulation able to predict how the force transmission affects the basket architecture.

This project has received funding from the ERC (GA No 646990 – NICHOID)

Keywords

Mechanotransduction; Nuclear pore complex; Bio-layer interferometry

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Abstract **#1048**

IMPEDANCE-OPTICAL DUAL-MODAL SENSOR AND DEEP-LEARNING-BASED IMAGE RECONSTRUCTION FOR 3D CELL CULTURE IMAGING

Zhe Liu[P][1], Pierre Bagnaninchi[2], Yunjie Yang[1]

[1] *Agile Tomography Group, School of Engineering, The University of Edinburgh, Edinburgh, UK*

[2] *Centre for Regenerative Medicine, Institute for Regeneration and Repair, The University of Edinburgh, 5 Little France Drive, Edinburgh EH16 4UU*

Corresponding author's email: zz.liu@ed.ac.uk

Electrical Impedance Tomography (EIT) is a prevailing imaging modality that can visualize conductivity distribution in a fast, non-radiative, non-intrusive manner [1]. Recently, it has been identified as a promising technique to monitor the electrical property distribution of 3D cell spheroids and infers their physiological properties throughout the growth [2, 3]. However, the EIT image reconstruction problem is severely non-linear and ill-posed, leading to the issue of low spatial resolution. In the past, the main efforts to improve the spatial resolution of the reconstructed conductivity images have been focusing on regularization-based single-modal methods [4-7] and regularization-based dual-modal or multi-modal methods [8-9]. The latter usually shows better performance but is still far from satisfactory. In this paper, a novel impedance-optical dual-modal sensor and a Multi-Scale Cross Feature Fusion Convolutional Neural Network (MSCFF-CNN) are proposed to improve the spatial resolution of EIT for 3D cell culture imaging. We validate the performance of the proposed method via simulation data and MCF-7 cell spheroids imaging experiments. The results suggest the combination of the dual-modal sensor and MSCFF-CNN outperforms the state-of-art regularization-based methods, which indicate the potential of a new structure-function joint imaging modality for 3D cell culture.

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Abstract **#1049**

QUANTITATIVE LONG-TERM LIVE IMAGING OF OVARIAN CANCER INVASION IN VITRO USING OPTICAL COHERENCE TOMOGRAPHY

Amelia Hallas-Potts[P][1], Zhe Liu[2], C Simon Herrington[1], Yunjie Yang[2], Pierre Bagnaninchi[3]

[1] Edinburgh Cancer Research Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh

[2] Agile Tomography Group, School of Engineering, The University of Edinburgh, Edinburgh, UK

[3] Centre for Regenerative Medicine, Institute for Regeneration and Repair, The University of Edinburgh, 5 Little France Drive, Edinburgh EH16 4UU

Corresponding author's email: zz.liu@ed.ac.uk

Ovarian cancer is a lethal gynecological disease, histological subtypes of ovarian cancer differ in their biology and clinical behavior. To better understand the behavior of ovarian tumors we are developing an in vitro model that can be monitored noninvasively in a time-lapsed manner to investigate the tumor invasion and eventually response to therapy over time.

Real time live imaging over 5 days was established on a commercial OCT system with novel image processing [1] using non-serous (NS) ovarian cancer cell lines TOV21G and A2780 seeded onto a collagen/fibroblast matrix. Using live OCT imaging we are able to reveal that TOV21G cells invade into collagen with a spindle-like morphology characteristic of sarcomatous tumor cell invasion whereas A2780 invade in clusters characteristic of epithelial tumor cells, in good agreement with histological observation, and how these different invasive patterns develop over time. We developed a pipeline of image processing algorithms that can quantitatively analyze the speed, depth and frequency of invasion over time. The Anisotropic Diffusion (AD) filtering [2] followed by Median filtering [3] can effectively remove speckle noise from the original OCT images and simultaneously preserve the edges. After locating the surface of the gel by Otsu's thresholding [4], a simplified one-dimensional Active Contour Model (s1-ACM) can locate the lower bound of the ovarian cancer layer. The combination of above three steps can automatically segment the ovarian cancer layer in this specific case and allow quantitative time-lapse imaging of its invasion.

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Abstract **#1050**

A NEXT GENERATION BIO-INSPIRED DEVICE FOR EFFECTIVE PERIPHERAL NERVE REGENERATION

Emmanuel Asare$_P$[1], Caroline S. Taylor[1], David A. Gregory[1], John W. Haycock[1], Ipsita Roy[1,2]

[1] *Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, UK*

[2] *National Heart and Lung Institute, Faculty of Medicine, Imperial College London, UK*

Corresponding author's email: easare1@sheffield.ac.uk

A major challenge in nerve tissue engineering and regeneration is the complexity of the nervous system and its associated biological processes. Peripheral nerve injuries (PNIs) less than 5 mm in length self-repair compared with those with wider gaps 1,2. Traditionally, PNIs of 20 mm or less have been repaired either by suturing nerves end-to-end, or by grafting. However, these techniques present surgical and patient recovery drawbacks, prompting the search for better technologies. Nerve guidance conduits (NGCs) have showed promise to mitigate these limitations but are still deficient in supporting satisfactory recovery of PNIs due to poor fit between the NGCs and the local environment. In this work, we have aimed to address this by developing a biomimetic nerve guidance prototype with improved physical and biochemical support for optimal PNI recovery. To achieve this goal, bacterial cellulose (BC) 3 , a versatile hydrogel, and a relatively unexplored family of natural, biocompatible, and bioresorbable polymers called Polyhydroxyalkanoates (PHAs) 4,5 have been produced via microbial fermentation. Our lab has recently optimized the production of PHAs resulting in about 10-fold increase in yield compared to the average values reported in literature. Additionally, a variety of constructs based on computer aided designs (CAD) ranging from multi-channel to internally grooved designs have been successfully 3D printed via fused deposition modelling (FDM) for their initial biocompatibility assessment with NG108-15 neuronal and Schwann cells. Preliminary results from our lab showed that PHAs supported superior neuronal proliferation and neurite extension over some of the commercially available state-of-the-art materials 5.

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Abstract **#1051**

ORAL DELIVERY OF ESOPHAGEAL EXTRACELLULAR MATRIX HYDROGEL MITIGATES DISEASE PROGRESSION IN A RAT MODEL OF ESOPHAGEAL ADENOCARCINOMA

Lindsey T Saldin$_P$[1], David G. Nascari[1], Xue Li[1], Ashten N Omstead[2], Lina M Quijano[1], Anastasia Gorbunova[2], Li Zhang[1], Paul Joseph[2], Blair A. Jobe[2], Vera S Donnenberg[3], Neill J Turner[1], Ali H Zaidi[2], Stephen F. Badylak[1]

[1] *McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA.*

[2] *Esophageal and Lung Institute, Allegheny Health Network, Pittsburgh, PA 15224, USA.*

[3] *Cardiothoracic Surgery, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA.*

Corresponding author's email: lsaldin@pitt.edu

Esophageal adenocarcinoma (EAC) has a 20% 5-year survival rate despite the current standard of care. EAC progresses in response to microenvironmental change, i.e., chronic gastric reflux and associated inflammation. A primary component of the microenvironment is the extracellular matrix (ECM), which directs cell phenotype by bi-directional crosstalk ("dynamic reciprocity"). The present study evaluated the effect of non-malignant esophageal ECM (eECM) hydrogel, delivered orally in vivo upon carcinoma in situ, without mucosal resection.

EAC developed for 24 weeks in a rat surgical model, and then eECM hydrogel, heterologous urinary bladder matrix (UBM-ECM) hydrogel, or buffer control, was delivered orally, twice daily, for 3 weeks (n=6-7/group). Gastrointestinal physicians who evaluated endoscopic videos before and after treatment were blinded. Five of 7 eECM treated animals, and 5 of 6 UBM-ECM treated animals, showed disease state reversion, while 4 of 6 buffer control animals showed no improvement. In the eECM and UBM-ECM responders, immunolabeling showed macrophages were modulated toward CD68+/TNFalpha+/IL1B+/CD206- compared to buffer control; suggesting a pro-inflammatory response at the tumor region is associated with improved outcome.

The direct effect of eECM and UBM-ECM hydrogel upon esophageal cancer cell (OE33) function and signaling was further investigated in vitro. Both eECM and UBM-ECM inhibited OE33 cell proliferation, and downregulated CD164/CXCR4 upstream of PI3K-Akt. However, only eECM decreased OE33 cell migration and BMP4/Snail2 signaling upstream of epithelial-mesenchymal transition, suggesting an enhanced tissue-specific effect.

The present study supports the use of eECM hydrogel as an alternative, safely tolerated, therapy for EAC that is based upon dynamic reciprocity.

Abstract **#1052**

AN IMAGE ANALYSIS-BASED WORKFLOW FOR BIOPRINTING OF ANATOMICALLY REALISTIC VASCULAR PATTERNS

Nicanor I. Moldovan$_P$[1], Rachel Cadle[2], Leni Moldovan[3]

[1] *Indiana Institute for Medical Research, Richard L. Roudebush VA Medical Center and Department of Ophthalmology, IUSM, Indianapolis, IN, USA*

[2] *Department of Biomedical Engineering, IUPUI, Indianapolis, IN, USA*

[3] *Department of Surgery, IUSM, Indianapolis, IN, USA*

Corresponding author's email: nimoldov@iupui.edu

For both in vitro applications and in vivo implantation, there is an enduring need to perfuse the tissue engineered constructs with vascular-like channels, optimized for distribution of nutrient-containing fluids. To address this 'vascular challenge', we explored the use for bioprinting of anatomically realistic patterns derived from macroscopic and microscopic images of human or animal vasculature. Moreover, to assign them organ-specific branching topology, and eventually a vessel size-appropriate cellular composition, we employed the image analysis program VESGEN 2D to assign branch generations to both arborescent and network microvascular fields. Here, we present workflows starting with primary image processing up to direct-write printing with model bioinks, of human ocular fundus, as well as rat mesentery and mouse intestine vascular patterns. For an even more accurate representation, we also generated hemispherical and cylindrical projections of the retinal and intestinal images, respectively. This work paves the way towards the incorporation within bioprinted constructs of anatomically realistic vasculature.

Abstract **#1053**

COMPUTATIONAL SIMULATION OF GLUCOSE EFFECTS FOR BIOFABRICATION OF CELL SPHEROIDS-BASED TUMOR MODELS

Nicanor I. Moldovan[P][1], David J. Bustamante[2], Elijah J. Basile[3], Brady M. Hildreth[3], Nathan W. Browning[3], Alex S. Jensen[4], Leni Moldovan[5], Horia I. Petrache[6]

[1] *Indiana Institute for Medical Research, Richard L. Roudebush VA Medical Center and IUPUI, Indianapolis, IN, USA*

[2] *Department of Biomedical Engineering, IUPUI, Indianapolis, IN, USA*

[3] *Department of Electrical and Computer Engineering, IUPUI, Indianapolis, IN, USA*

[4] *Department of Computer Science, IUPUI, Indianapolis, In, USA*

[5] *Department of Surgery, IUSM, Indianapolis, IN, USA*

[6] *Departments of Physics, IUPUI, Indianapolis, IN, USA*

Corresponding author's email: nimoldov@iupui.edu

In vitro tumor models consisting of cell spheroids are increasingly used for mechanistic studies and pharmacological testing. However, unless vascularized, the availability of nutrients such as glucose to deeper layers of multicellular aggregates is limited. In addition, recent developments in 'scaffold-free' (cells-only) biofabrication, allow the creation of more complex spheroid-based structures, further exposing the cells within these constructs to nutrient deprivation. To explore the impact of glucose availability on such tumor-like structures, we used the computer simulation platform CompuCell3D. By monitoring the types of cells and the distance between spheroids centers of mass, we studied how binary- and multi-spheroid fusions are impacted by glucose availability. At limiting glucose concentrations mimicking hypoglycemia we noted an abrupt collapse of the tumor spheroids, a process amplified by the contact with normal cell spheroids. At higher glucose concentrations, we found an increased intermixing of cancerous cells, anti-phase oscillations between proliferating and quiescent tumor cells and a structural instability of fusing tumor spheroids, leading to their fragmentation. In a model of tumor microenvironment composed of normal cell spheroids fusing around a tumoral one, the competition for glucose lead to either the tumor's disappearance, or to its steady expansion. Moreover, the invasion of this microenvironment by individual tumor cells also directly depended on the available glucose. In conclusion, we demonstrate the heuristic value of computational simulations for anticipating the properties of biofabricated tumor models, and in generating testable hypotheses regarding the relationship between cancer, nutrition and diabetes.

Abstract **#1054**

HARNESSING NATIVE EXTRACELLULAR MATRIX FOR BONE REGENERATION: A COMPREHENSIVE STUDY

Fanny Blaudez[P][1,2]

[1] *Griffith university*

[2] *University of Queensland*

Corresponding author's email: fanny.blaudez@gmail.com

Bone regeneration in the craniofacial area is challenging and tissue engineering strategies have been developed in order to increase efficacy and predictability of osteogenesis. However, these approaches rely on the utilisation of autologous cell sources, associated with high cost, hence detrimental for economically viable clinical translation. In this study, we explored the concept of extracellular matrix (ECM) decoration to enhance the bioactivity of polycaprolactone (PCL) scaffolds manufactured by melt-electrowriting. To this end, scaffolds of various pore sizes were seeded with human primary osteoblasts and cultured for 1, 2 or 4 weeks to allow bone-specific ECM deposition. Mature constructs were subsequently decellularised, resulting in an acellular ECM-decorated scaffold. Matrices were characterised before and after decellularisation and were recellularised to characterise the in vitro behaviour of human primary osteoblasts. Bone healing potential was then assessed in vivo in a rodent calvarial model. The various culture periods demonstrated that ECM composition was significantly impacted by the scaffold porosity and the culture time. ECM-decoration was observed at early time points, gradually mineralising and leading to obstruction in smaller pores. This influenced cellular infiltration and metabolic activity of reseeded cells. In vivo,bone regeneration was enhanced by specimen cultured 1 week prior to decellularisation, indicating that short/mid-term in vitro decoration is beneficial for osteogenesis. This research investigated the influence of in vitro maturation on the composition and osteogenic capacity of ECM-decorated scaffolds and demonstrated that the optimisation of culture time prior to decellularisation is essential to maximise cell infiltration, and differentiation towards the osteogenic lineage.

Abstract **#1055**

BIOPRINTING OF PATIENT-DERIVED IN VITRO CHOLANGIOCARCINOMA TUMOR MODEL

Yuan Pang[P][1], Shuangshuang Mao[1], Yilei Mao[2], Huayu Yang[2], Feihu Xie[2], Wei Sun[1,3]

[1] *Tsinghua University*

[2] *Peking Union Medical College (PUMC) Hospital*

[3] *Drexel University*

Corresponding author's email: pangyuan31@tsinghua.edu.cn

Towards the development of in vivo-mimicking tumor model for extensive study of tumorigenesis and establishment of personalized therapy, patient-derived primary tumor cells were employed in this work for three-dimensional (3D) bioprinting. Cholangiocarcinoma cells isolated from patient were bioprinted using a composite hydrogel system of gelatin-alginate-MatrigelTM into pre-designed grid architecture. Cholangiocarcinoma cells were observed to process a colony forming ability with high survival rate and active proliferation. Expressions of tumor markers, cancer stem cell markers, matrix metalloproteinase protein, index of tumor fibrosis, index of liver function, and epithelial-mesenchymal transition (EMT) regulatory proteins confirmed the development of the invasive and metastatic phenotype of the cholangiocarcinoma cells in the 3D printed tumor microenvironment. Similar results were obtained in anti-cancer drug resistance of the cholangiocarcinoma cells in the 3D bioprinted construct that demonstrated stem-like properties, which suggested the promising potential of current 3D printed tumor model in the development of personalized therapy, especially for discovery of more conducive targeted drugs.

Abstract **#1056**

SYNERGISTIC PHOTOTHERAPY AND TUMOR IMAGING VIA HIGHLY BIOCOMPATIBLE NEAR-INFRARED NANOMATERIALS

Pravin Bhattarai[1,2], Xiaolong Liang[3], Yunxue Xu[1], Zhifei Dai[1]

[1] *Department of Biomedical Engineering, College of Engineering, Peking University, Beijing 100871, China*

[2] *Phutung Research Institute, Kathmandu, Nepal*

[3] *Department of Ultrasonography, Peking University Third Hospital, Beijing 100191, China*

Corresponding author's email: pravin.sirish@gmail.com

The design and synthesis of multifunctional nanoparticles (NPs) for drug delivery, imaging, and phototherapy has been investigated widely in past several years. However, inadequate drug loading, limited imaging capabilities in the near-infrared fluorescence region (NIRF), and complete tumor eradication is still a major challenge. To resolve aforementioned issues, a most commonly used photosensitizer, porphyrin, was first chemically modified to porphyrin grafted lipids (PGLs) having a hydrophobic head and a long hydrophilic tail. Similar to liposomes, such PGL NPs self-assembled together with a lipophilic cyanine dye, DiR resulting in a new combination NPs (PGL-DiR NPs) of average size 156 nm. Importantly, this technique addresses issues related to limited loading capacities of hydrophobic porphyrins and DiR as well. In addition, the strategic combination of PGL-DiR NPs was further utilized to trigger a light-activatable photoresponsive (photothermal therapy, PTT; photodynamic therapy, PDT) properties. The amount of DiR loading was optimized to induce quenching of singlet oxygen (1O2) from PGL followed by subsequent recovery after irradiation of appropriate laser. Such activatable NPs are of key importance to realize synergistic phototherapy i.e. PTT and PDT without affecting nearby normal cells. After intravenous administration of PGL-DiR NPs in a PC3 tumor xenograft mice, a high accumulation in tumor until 24 hrs was clearly evident via NIRF imaging. With the successive laser irradiation, these NPs effectively suppressed the tumor growth synergistically while compared to monotherapies such as PTT or PDT alone.

Keywords

Porphyrin; Cyanine; Photodynamic therapy

Abstract **#1057**

3D MICROFABRICATED SCAFFOLDS INFLUENCE MESENCHYMAL STEM CELL MIGRATION AND DNA ORGANIZATION.

Emanuela Jacchetti[1], Bianca Barzaghini[1], Federica Carnevali[1], Ramin Nashei[1], Tommaso Zandrini[1,2], Roberto Osellame[2], Giulio Cerullo[2], Stephana Carelli[3], Manuela Teresa Raimondi[1]

[1] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy.

[2] Istituto di Fotonica e Nanotecnologie (IFN)-CNR and Department of Physics, Politecnico di Milano, Milano, Italy.

[3] Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", L. Sacco Department of Biomedical and Clinical Science, University of Milan, Milan, Italy.

Corresponding author's email: emanuela.jacchetti@polimi.it

Introduction: 3D biomaterials are increasingly being used to control stem cell fate in vitro. To modulate cell-substrate interaction we developed an isotropic 3D-microstructured substrate nanoengineered via two-photon-laser polymerization [1]. We studied its influence on cell mechanotransduction pathway modulation, gene expression and cell migration.
Methods: Scaffolds are microfabricated structure composed by cubic pore with dimension 15x15x15µm^3. Results were compared with those obtained on 2D glass-coverslip. 10^4MSCs were cultured for 24 hours, for each experiment. Cells were processed for immunofluorescence analysis to characterize cytoskeleton and chromatin organization. Timelapse microscopy and DNA methylation were performed to analyse cell migration and DNA activity.
Results: MSC grown into our 3D-substrates show:
1. Remodulation of the nuclear shape from a thin disk to a roundish ellipsoid.
3. Reorganization of the DNA: DNA methylation is lower and euchromatin is more homogeneously distributed.
4. Strong reduction in cell capability to migrate.
Discussion and conclusion. Results show that our 3D-microstructured scaffolds affect migratory cell behaviour and gene activity, suggesting that MSCs grown in this culture condition could maintain cellular stemness. We are evaluating cell potency by analysing the activity of fundamental genes such as SOX2, NANOG, OCT4.

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Abstract **#1058**

RADIALLY AND CIRCULARLY ALIGNED FIBERS TO GUIDE STEM CELL GROWTH: AN ADDITIVE ELECTROSPINNING APPROACH FOR EARDRUM REGENERATION

Lorenzo Zavagna[P][1,2], Bahareh Azimi[1,2], Claudio Ricci[1,3], Stefano Berrettini[1,3], Stefano Linari[4], Serena Danti[1,2]

[1] *Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM), 50121 Florence, Italy*

[2] *Department of Civil and Industrial Engineering, University of Pisa (Unipi), Pisa, Italy*

[3] *OTOLAB, Department of Surgical, Medical, Molecular Pathology and Emergency Medicine, University of Pisa, Pisa, Italy*

[4] *Linari Engineering s.r.l., Via Umberto Forti 24/14, 56121, Pisa, Italy*

Corresponding author's email: lorenzo@zavagna.it

The first step of the hearing process takes place at the tympanic membrane (TM), a thin and tough, concave-shaped membrane composed of three different layers [1], which is responsible for transmitting air pressure weaves collected in the ear canal into vibrations of the ossicular chain [2]. The pars tensa is considered the main actor of the TM acoustic response and is composed of two differently oriented layers of collagen fibers: radial and circular [1, 3].

We developed a specific electrospinning collector and setup able to produce an anatomically consistent scaffold, composed of two layers, deposited one onto each other, of ultrafine fibers with radial and circular orientations. The polymer used was poly(ethylene oxide terephthalate)/poly(butylene terephthalate) [2, 4]. Finite Element Analysis of the electrostatic field was performed to design the optimal collector shape (i.e. able to generate the correct electric field spatial distribution) to reach the desired alignments. The fiber diameter distribution and morphology were characterized by scanning electron microscopy. Radial fibers had 1.00 ± 0.15 µm, whereas circular fibers had 0.8 ± 0.4 µm size. All the main anatomical features of the TM (e.g. thickness variability) were successfully reproduced in this scaffold. Cytocompatibility studies performed with human mesenchymal stromal cells revealed excellent cellular adhesion and proliferation. Fluorescence microscopy observation of the cytoplasm and nuclei indicated that the cell grew in an aligned fashion, consistently with the underlying fibers.

The obtained fiber size and alignment revealed potential for eardrum regeneration.

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Abstract **#1059**

NANOCLAY GELS FOR SAFE AND EFFICIENT BMP-2 BONE INDUCTION – AN ANALYSIS OF NANOCLAY AND BMP2 BIODISTRIBUTION IN VIVO

Yang-Hee Kim[P][1,2], Agnieszka Janeczek[2], Richard O.C Oreffo[1,2], Jonathan I Dawson[1,2]

[1] *University of Southampton*

[2] *Renovos®*

Corresponding author's email: Yanghee.Kim@soton.ac.uk

Nanoclays have attracted attention in the regenerative medicine field for their self-assembly properties, protein interactions, and intrinsic osteogenic bioactivity. While nanoclay-localised BMP-2 displays bone formation ability [1,2], to date, no studies have explored whether nanoclays and BMP-2 molecules distribute to other organs/tissues following implantation and during biodegradation.

We have evaluated the biodistribution of nanoclays and BMP-2 by tracking nanoclay elements and BMP-2 molecules specifically in the blood and organs such as heart, kidney, liver, lung, spleen, stomach, and bladder. Nanoclay gels with/without fluorescently labelled BMP-2 were subcutaneously injected to female MF-1 wild type mice. Over the course of 8 weeks BMP2 biodistribution was tracked using an in vivo imaging system (IVIS) alongside micro-computed tomography (μ-CT) and histological analysis of new bone formation. Nanoclay elements in harvested tissues were quantified using inductively coupled plasma mass spectrometry (ICP-MS)

In the absence of nanoclay, BMP2 rapidly dispersed to below the detection threshold after 4 hours and no bone formation was observed at week 8. In contrast, BMP-2 with nanoclay was detectable even at 8 weeks and induced ectopic bone. Nanoclay was progressively removed from the implant site correlating with cellular invasion and replacement by new bone tissue. Nanoclay did not accumulate in lung, heart, blood or stomach and temporal distribution in liver and bladder was consistent with excretion via liver and urinary systems. These studies demonstrate that nanoclay can maximise bone formation efficacy of BMP-2 with no side effects on internal organs by BMP-2 localisation, offering a safe delivery approach for this biologic.

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Abstract **#1060**

OPTIMISING HUMAN BONE EXTRACELLULAR MATRIX DERIVED HYDROGELS FOR BONE TISSUE ENGINEERING – AN ANALYSIS OF PROCESSING PARAMETERS

Yang-Hee Kim[P][1], Gianluca Cidonio[1,2], Juan Aviles Milan[1], Richard O.C Oreffo[1], Jonathan I Dawson[3]

[1] *University of Southampton*

[2] *Istituto Italiano di Tecnologia*

[3] *University of Southampton*

Corresponding author's email: Yanghee.Kim@soton.ac.uk

Decellularised tissue provides a promising biological material for tissue regeneration. A central feature of decellularised tissue is the elimination of the cellular components from the native tissues while retaining the complex compositions and architecture of extracellular matrix (ECM) [1]. While decellularised ECM hydrogels from bovine and porcine bone tissues have been examined in the field of tissue regeneration [2], to date, no studies have sourced or evaluated decellularised matrices from human bone tissue.

The objective of this study was to evaluate the hypothesis that physiochemical and biological properties of ECM hydrogel derived from demineralised and decellularised human bone matrix could be controlled through modulation of bone powder size and digestion time for enhanced bone repair. Trabecular bone from human femoral head samples following hip arthroplasty were collected and demineralised human bone powders at various sizes (45-250 µm, 250-1000 µm, and 1000-2000 µm) prepared. After decellularisation, the powders were treated with pepsin solution for 3, 5, and 7 days. The concentration of proteins and gelation strength significantly increased as bone powder size decreased and digestion time increased. Evaluation of cultured human bone marrow-derived stromal cells on the ECM hydrogel derived from 45-250 µm bone powder size and treated for 7 days exhibited significantly enhanced cell proliferation and lineage differentiation. Taken together, a defined digestion time and powder size are crucial factors for generating human bone ECM hydrogels with a high concentration of proteins and rheological property for augmentation of bone repair.

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Abstract **#1061**

TOWARDS THE JOINT ON A CHIP: DIFFERENTIATING DIRECTLY INTERFACED MICRO-TISSUES TO MIMIC OSTEOARTHRITIC CARTILAGE-SUBCHONDRAL INTERFACE IN A MECHANICALLY ACTIVE ENVIRONMENT

Andrea Mainardi[P][1,2,3]

[1] *Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milan, Italy*

[2] *Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland*

[3] *Department of Biomedical Engineering, University of Basel, Allschwil, Switzerland.*

Corresponding author's email: andrea.mainardi@polimi.it

Osteoarthritis (OA), the most diffused musculoskeletal pathology, is a whole joint disease affecting articular cartilage (AC) but also subchondral layers. Most OA in vitro models, however, do not account for the multiplicity of tissues affected, nor for the mechanically active joint environment. In this work we present an Organ on chip model of OA enabling the subjection of multi-layer tissues to cyclical compression.

Starting from a cartilage on chip model [1], human articular chondrocytes laden hydrogels were differentiated into AC. Hypertrophic cartilage (HC) was, instead, obtained from MSCs. A direct interface between co-cultured 3D micro-constructs was achieved through removable PDMS molds permitting sequential substrates injections. [2]. Aggrecan and Collagen type-II characterized the AC matrix, while the HC matrix, still Col2a1 positive, was also rich in collagen type-X and calcium deposits, hallmarks of the hypertrophic tissue. The culture chamber was coupled with a mechanical actuation compartment through a pressure sensitive adhesive based mechanism. This way vertically stacked constructs can be exposed to defined levels of mechanical compression during culture, allowing for the study of biomechanics role in OA onset. Indeed, these results could bring to a more complex and representative in vitro representation of OA joints, easing the investigation of the causes leading to OA (e.g. mechanical injury), but also of the role of interplays between different tissues (e.g. the advancement of HC into AC). Such a model could be a powerful tool for both basic research and drug screening.

Acknowledgments

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Abstract **#1062**

AICAR AND NAM SYNERGISTICALLY ATTENUATE SENESCENCE-ASSOCIATED CHANGES IN MESENCHYMAL STEM CELLS: THE INTERPLAY OF AUTOPHAGY AND MTORC1

Mohammadhossein Khorraminejad-Shirazi[P][1,2], Mahsa Sani[3], Tahereh Talaei-Khozani[4], Mohammadreza Dorvash[2], Malihe Mirzaei[5], Mohammad Ali Faghihi[5,6], Ahmad Monabati[7,8], Armin Attar[9]

[1] Student research committee, Shiraz University of Medical Sciences, Shiraz, Iran

[2] Cell and Molecular Medicine Student Research Group, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[3] Tissue Engineering Department, School of Advanced Medical Science and Technology, Shiraz University of Medical Science, Shiraz, Iran

[4] Tissue Engineering Lab, Department of Anatomical Sciences, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[5] Persian BayanGene Research and Training Center, Shiraz University of Medical Sciences, Shiraz, Iran

[6] Center for Therapeutic Innovation, Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, FL, USA

[7] Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[8] Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[9] Department of Cardiovascular Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding author's email: hkhorraminejad@gmail.com

Tissue engineering has yet to reach its ideal goal, i.e. creating profitable off-the-shelf tissues and organs (1,2). One of the major challenges of this field that affects the outcome of the cell transplantation is the in-vitro aging and replicative senescence of the cell source #. In the present work, Passage 5 (P5) adipose-derived mesenchymal stem cells (MSCs) were treated with control media, nicotinamide (NAM), AICAR, or the combination of AICAR+NAM till P10. Proliferative capacity, senescence-associated changes, multi-lineage differentiation potential, CDK-inhibitors -P16 and P21-, ROS, and apoptotic markers were compared between cells of P5 and P10. Furthermore, we studied the effects of AICAR and NAM on mTORC1 activity, and autophagy.

Our results showed that MSCs treated with NAM, AICAR, or both demonstrated an increase in proliferation and osteogenic differentiation potential, which was amplified in the group receiving both. AICAR or NAM treatment resulted in reduced expression of β-galactosidase and decreased accumulation of dysfunctional lysosomes. Also, NAM or AICAR+NAM significantly reduced the cellular ROS in aged MSCs. Moreover, AICAR and NAM administration attenuated mTORC1 activity and boosted autophagy.

In general, inhibition of mTORC1 by AICAR and NAM upregulates autophagy, retains MSCs' self-renewal capacity, and delays senescence of MSCs after prolonged in-vitro culture. Furthermore, our findings showed that concomitant use of AICAR and NAM shows a synergistic effect on the attenuation of cellular senescence. Our findings provide a viable option to slow down the in-vitro aging of the stem cells and conquer aging as a limiting factor in tissue engineering.

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Abstract **#1063**

STABILITY OF CALCIUM PHOSPHATE COATED MELT ELECTROWRITTEN (MEW) SCAFFOLDS AND OSTEOGENESIS

Naghmeh Abbasi$_P$[1]

[1] *School of Dentistry and Oral Health, Griffith University, Gold Coast Campus, Southport, Queensland, 4215, Australia*

Corresponding author's email: naghme.k@gmail.com

CaP coating on melt electrowritten (MEW) substrates demonstrated itself as a potential candidate for bone regeneration due to mimick the natural bone tissue. It increased the osteoblast cells - implanted scaffolds interactions and improved bone ingrowth. This study aims to evaluate the stability and structural properties of CaP coating on melt electrowritten PCL scaffolds following pre-treatments of Ar- O2 plasma and NaOH solution to improve the wettability.
The plasma-treated fibers were uniformly coated after one hour. The surface wettability enhanced through the mineralization of both plasma and NaOH pre-treated scaffolds. Mechanical properties of the scaffolds degraded through the plasma and NaOH treatment. However, tensile stability was improved following mineralization in plasma-treated scaffolds due to the smaller crystal size and a dense CaP layer, which leads to the higher solubility. We demonstrated that the plasma pre-treated mineralized surface is stable enough to be potentially useful for the further development of bone regeneration. and could successfully direct cells toward an osteogenic lineage with resulting mineralization.

Keywords

Melt electrowriting; Calcium phosphate coating; Plasma treatment

Abstract **#1064**

MODULATING MOLECULAR INTERACTION BETWEEN HYDROGEL COMPONENT AND GROWTH FACTOR DICTATE EFFECTIVE BONE FORMATION

Oommen Varghese[P][1], Maruthibabu Paidikondala[1], Jöns Hilborn[2], Oommen P Oommen[3]

[1] Translational Chemical Biology, Polymer Chemistry Division, Department of Chemsitry, Ångström Laboratory, Uppsala University, Sweden

[2] Polymer Chemistry, Department of Chemsitry, Ångström Laboratory, Uppsala University, Sweden

[3] Bioengineering and Nanomedicine Lab, Faculty of Medicine and Health Technologies, Tampere University and BioMediTech Institute, 33720, Tampere, Finland

Corresponding author's email: oommen.varghese@kemi.uu.se

The use of injectable gels derived from the natural extracellular matrix (ECM) to deliver proteins has attracted much interest in the field of regenerative medicine. We have previously demonstrated that hyaluronic acid (HA) hydrogel could be used for efficient delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) and induce bone formation within 6 weeks when injected below the rat periostium.[1] Though rhBMP-2 is very potent for inducing bone formation, the dose dependent toxicity is a major concern.[2] This is mainly due to supraphysiological dose that is clinically used since the collagen-based BMP-2 carrier is inefficient and does not stabilize rhBMP-2 in vivo. We have devised a new strategy to stabilize BMP-2 and improve bone formation with a minimal dose of the protein.

To develop HA-based hydrogel materials we tested two types of crosslinking chemistry, namely hydrazone and thiol-Michael coupling reaction.[3] These materials were evaluated to deliver rhBMP2 in vitro as well as in vivo in a rat ectopic model. Since the ECM in the living system is known to stabilize growth factors by electrostatic and hydrophobic interactions, we also developed 'acidic' and 'neutral' form of HA by changing the protonation/deprotonation of the carboxylic groups and evaluated the protein interaction with HA at different pH.[4] Such manipulation of charge of the biopolymer had a profound impact on protein release at physiological pH as determined using cell-based ALP assay. The effect of BMP-2 stability and function was quantified in vivo by measuring the bone volume after 6 weeks of implantation.

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Abstract **#1065**

THE HYBRID BILAYER BIOMATRIX OF FUNCTIONALIZED CELLULOSE HYDROGEL LAYERED ON COLLAGEN SCAFFOLD FOR WOUND HEALING APPLICATION

Atiqah Salleh[P][1], Fauzi Mh Busra[1]

[1] *Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Kuala Lumpur, Malaysia*

Corresponding author's email: atqhsalleh@gmail.com

Tissue engineering technologies are well-established alternative approaches currently available for wound applications either chronic or acute injuries. However, the success rate post-implantation is still low due to high susceptibility of severe bacterial infections. Thus, a relevant improvement still required to accelerate wound healing for better recovery. This study aimed to fabricate a bilayer hybrid scaffold containing ovine collagen type-I (OCT-I) sponge and functionalized-cellulose hydrogel (CH) at top layer. Briefly, the OCT-I was freeze-dried and crosslinked with 0.1% of genipin (GNP), natural crosslinking agent, for 6 hours. Meanwhile, the CH was prepared, and integrated with graphene oxide-silver nanoparticles (GO-Ag) prior to hybridization. The fabricated hybrid biomatrix further investigated for 3D-microstructure, biodegradability, surface wettability, anti-bacterial activity, and its biocompatibility towards human skin cells. The degree of crosslinking between GNP and OTC-I sponge exceed 90% which showed that GNP is favorable as crosslinker. The results revealed that GNP crosslinked OTC-I scaffold showed better physicochemical and mechanical properties compared with the non-crosslinked OTC-I scaffold. Even though, the non-crosslinked OTC-I scaffold great swelling ability (1638.89 ± 1.76%) than GNP crosslinked OTC-I scaffold (1214.56 ± 1.05%) in 24h, GNP crosslinked had lower biodegradability (18.32 ± 8.53%) and good hydrophilicity (77.54 ± 1.62°). The CH also showed great anti-antimicrobial activity as well as good physicochemical properties. Moreover, the fabricated bilayer scaffold showed good cell adhesion and proliferation with human epidermal keratinocytes and human dermal fibroblasts. In conclusion, the fabricated bioscaffold potentially a good candidate for the wound healing application.

Keywords

Collagen biomatrix; Cellulose hydrogel; Hybrid bioscaffold

Abstract **#1066**

PEPTIDE/GRAPHENE OXIDE HYBRID HYDROGELS DECORATED WITH TGF-B3 AS 3D INJECTABLE SCAFFOLDS FOR NUCLEUS PULPOSUS TISSUE ENGINEERING

Cosimo Ligorio[P][1,2], Aravind Vijayaraghavan[1], Judith Hoyland[3], Alberto Saiani[1,2]

[1] *Department of Materials, School of Natural Sciences, The University of Manchester, Manchester, UK*

[2] *Manchester Institute of Biotechnology, The University of Manchester, Manchester, UK*

[3] *Division of Cell Matrix Biology and Regenerative Medicine, School of Biological Sciences, The University of Manchester, Manchester, UK*

Corresponding author's email: cosimo.ligorio@manchester.ac.uk

A recent strategy to design functional injectable hydrogels relies on the incorporation of nanofillers into the hydrogel matrix to add functionalities [1,2]. Intervertebral disc degeneration (IVDD), initiating in the nucleus pulposus (NP), represents a clinically relevant example where injectable systems could bring significant benefits. Here we designed an injectable cell carrier for NP repair by exploiting graphene oxide (GO) as nanofiller of FEFKFEFK (F8) peptide hydrogels and as delivery cargo for transforming growth factor beta-3 (TGF-β3).

Hydrogel nanocomposites were prepared with non-covalent synthesis and hydrogel structure was assessed via spectroscopy and microscopy techniques (FTIR, AFM and TEM), while gel stiffness and injectability were measured via oscillatory rheometry. Bovine NP cells were cultured in the nanocomposites by supplying TGF-β3 exogenously, endogenously (10 ng/ml) or by exploiting GO as drug delivery platform. Cell viability, metabolic activity, gene and protein expression of encapsulated NP cells were assessed.

Incorporation of GO promoted strong peptide-GO molecular interactions leading to storage moduli (~12.8 kPa) comparable to the human NP (~10 kPa). Nanocomposites showed ease of injectability, with a minimal release of TGF-β3 over 3 weeks (<3%). Having TGF-β3 complexed on GO led to an increased gene expression and deposition of characteristic NP matrix components after 21 days of 3D cell culture, compared with peptide only or with nanocomposites having TGF-β3 as free molecule. Collectively, the triad peptide-GO-TGF-β3 provided a suitable cocktail of mechanical and biochemical cues to promote cell proliferation and enhanced matrix deposition, harbouring therefore significant potential as functional biomaterial for IVD repair.

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Abstract **#1067**

A HYBRID APPROACH TO ENGINEER THE TENDON SYNOVIAL SHEATH FOR PREVENTION OF POST-OPERATIVE ADHESIONS

Angela Imere[P][1], Jason Wong[2,3], Marco Domingos[4], Sarah Cartmell[1]

[1] Department of Materials, School of Natural Sciences, The University of Manchester, Manchester, UK

[2] Blond McIndoe Laboratories, School of Biological Sciences, The University of Manchester, Manchester, UK

[3] Department of Plastic Surgery & Burns, Wythenshawe Hospital, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK

[4] Department of Mechanical, Aerospace and Civil Engineering, School of Engineering, The University of Manchester, Manchester, UK

Corresponding author's email: angela.imere@manchester.ac.uk

Clinical treatments for tendon lacerations can be compromised by adhesion formation[1] due to tendon synovial sheath disruption and aberrant healing. One of the most promising anti-adhesion approaches relies on the introduction of a biomembrane as physical barrier for adhesion-forming cells whilst regenerating the tendon synovial sheath[2]. Here, we propose a novel hybrid approach that combines electrospinning and 3D bioprinting techniques to produce a bilayer biomembrane for restoration of tendon synovial sheath integrity and prevention of post-operative adhesions.

Polymeric meshes were prepared by electrospinning, using 10% w/v poly(ε-caprolactone) (PCL) in 1,1,1,3,3,3,-hexafluoro-2-propanol. Samples were imaged using SEM and mechanically tested under tension. Migratory ability of human dermal fibroblasts through the mesh was studied using a double chamber system. Alpha4® peptide hydrogel was loaded with HIG-82s (synoviocytes) and printed using a 3D Discovery bioprinter. Cell viability and metabolic activity were evaluated over 4 weeks. Production of extracellular matrix (ECM) proteins and hyaluronic acid (HA) was assessed using histology and immunocytochemistry. Membrane's frictional properties on porcine tendon were evaluated by oscillatory rheology.

Results show that the nanofibrous mesh (Mean=0.25µm) can withstand the forces generated in vivo and prevents fibroblast infiltration in vitro due to its small pores (<3µm). 3D bioprinting allows for accurate spatial distribution of the hydrogel phase without affecting synoviocyte viability and proliferation. Moreover, Alpha4® supports cells to produce an ECM that mimics the native tissue and possesses anti-adhesive properties. In conclusion, the biomembrane acts as physical barrier and has the potential to restore the native content of HA for long-term lubrication.

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Abstract **#1068**

SCALABILITY OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED KIDNEY TISSUE ORGANOIDS, A STEP TOWARDS CLINICAL APPLICATION

Loes Wiersma[P][1], Cathelijne van den Berg[1], Ellen Lievers[1], Ton Rabelink[1]

[1] *LUMC*

Corresponding author's email: l.e.wiersma@lumc.nl

The generation of human induced pluripotent stem cells (hiPSCs) has opened up a world of opportunities for stem cell based therapies in regenerative medicine. These cells have the unique potential to become any cell of the human body. Worldwide there are many people suffering from reduced kidney function, and thousands are in need of a donor kidney. Currently there are several human kidney organoid protocols available that generate organoids containing kidney structures. In our lab we generate kidney organoids from hiPSCs that contain glomerular, proximal tubular and more distal tubular structures. These kidney organoids are relatively small ranging up to 4 mm in diameter in vitro. Here we show a scalable and reproducible method to increase the size of the organoid up to 1.5 cm in diameter. Confocal microscopy showed that all structures, e.g. NPHS1, LTL and ECAD for glomerular, proximal and distal tubular structures respectively remained evenly distributed throughout the entire sheet. Analysis of these sheet organoids shows that they can attain at least 14.000 NPHS1 positive glomerular structures. Upon transplantation in immunodeficient mice the organoids became vascularized and further matured. These sheet organoids also showed reuptake of injected low-molecular mass dextran molecules in the tubular structures upon filtration in the glomeruli. The scalability of the procedures is an important step forward in the translation of these differentiation protocols to future clinical applications in the development of a bioengineered kidney.

Keywords

Kidney organoid; Scalability

Abstract **#1069**

3D BIOPRINTING TECHNOLOGY FOR BUILDING MICROPHYSIOLOGICAL SYSTEMS OF HUMAN CANCER

Hee-Gyeong Yi[1]

[1] *Department of Rural and Biosystems Engineering, College of Agricultural Science, Chonnam National University*

Corresponding author's email: hgyi@jnu.ac.kr

Microphysiological systems of human cancer are emerging platforms for better cancer research. Conventional cancer models have failed in capturing the underlying mechanisms of how cancer reacts to the current anti-cancer approaches due to the lack of similarities with the original cancer of the patient. The challenge is to model a complex and heterogeneous cancer environment where every element contributes to cancer hallmarks development. Recently, biofabrication technologies have been advanced to spatially control multiple types of cells and biomaterials to mimic the native physiology of tissue and organs. In particular, 3D bioprinting has beauty in manipulating the 3D anatomical structure of cancerous tissue through a layer-by-layer process that stacks up cells and bioinks following precisely designed sequences. The 3D bioprinted microphysiological system of human cancer has recapitulated the pathology and aggressiveness of original cancer. In addition, 3D bioprinting has shown the possibilities in modeling various types of cancers for future cancer research.

Abstract **#1070**

UNDERSTANDING C2C12 CELLS' SMAD SIGNALLING UPON PRESENTATION OF BMP 2/7 CHIMERA OR INDIVIDUAL BMPS IN A TEMPORAL OR SPATIALLY CONTROLLED SYSTEM

Siti Hawa Ngalim[P][1], Rhisita Nath[1], Elisabetta Ada Cavalcanti-Adam[2]

[1] *Universiti Sains Malaysia*

[2] *Max-Planck Institute for Medical Research*

Corresponding author's email: siti.hawa.ngalim@usm.my

C2C12 cell line (mouse myoblast cell line) that differentiates into myotubes (muscle cell) upon confluency or transdifferentiates into osteoblasts (bone cells) upon Bone Morphogenetic (BMP) 2 induction. Such characteristic has made C2C12 a stem cell-like cell line that is ideal for myotubes/osteoblast research model. Current literature shows BMP2 and BMP7 heterodimers are present and important in the early mouse embryo and Xenopus embryo development (1-2). It is not yet clear how spatially controlled BMP2/7 chimera presentation, in soluble and immobilised forms, influence C2C12 cells morphology and cell signalling any differently. As a start, we compare Smad 1/5/9 signalling activity when C2C12 cells were presented to a soluble form of the BMP: sBMP2/7 chimera, sBMP2 or sBMP7 alone. Only 7.5nM BMP2 and BMP2/7 chimera while 22.5nM BMP7 were needed to exert significant differences (**P < 0.005) in Smad 1/5/9 activities with their respective negative control within 45 mins. Next, diblockcopolymer micelle nanolithography (BCMN) technique was used to generate spatially controlled of approximately 8 nm gold nanoparticles and they were biofunctionalised after. Two different types of spacings were generated: 49.00 +/- 1.95 nm with an order of 0.5, and 95.82 +/- 0.92 nm with an order of 0.70. Quantitation of BMP immobilisation via mu N-hydroxysuccinamide reaction was done on gold monolayer of quartz crystal microbalance – dissipation (QCM-D). Next, C2C12 cells' morphology on the immobilised BMPs, Smad 1/5/9 expressions and involvement of certain integrin subunits will be tested.

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Abstract **#1071**

ELECTROSPUN POLY (E-CAPROLACTONE)/TIO2 NANOCOMPOSITE SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATION; IN-VITRO STUDY

seyedeh shima mohammadiP[1], seyedeh sara shafiei[1]

[1] *Department of Stem Cells and Regenerative Medicine, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*

Corresponding author's email: seyedehshima@icloud.com

Electrospinning is a widespread technique used in the tissue engineering field, due to the fabrication of nanoscale/microscale fibrous structures similar to the native extracellular matrix morphology. Polycaprolactone (PCL) is biocompatible aliphatic polyester with high potential usage in medical applications. On the other hand, Gelatin (GE) type A is used as an additive in order to increased nanofibers degradation and hydrophilicity, and also improve solution viscosity. In this study, nanofibrous scaffolds based on PCL/Ge containing TiO2 nanoparticles were fabricated by electrospinning method.

The main objective of this study is to investigate the effect of Tio2 NPs on human mesenchymal stem cell-derived bone marrow, in terms of cytotoxicity, releasing reactive oxygen specious (ROS), adhesiveness, and survival.

the advantageous properties of Tio2 NPs such as light weight, bioactivity, biocompatible, antibacterial, corrosion protecting have made it an interesting material to be considering in compositing with polymers.

The results demonstrated that the addition of TiO2 NPs improved the mechanical strength, hydrophilicity, and cellular attachments of scaffolds.

Keywords

tissue engineering; Electrospinning; Tio2 nanoparticles

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Abstract **#1072**

ISOLATION AND CHARACTERIZATION OF CRAB HEMOLYMPH EXOSOMES AND ITS EFFECTS ON BREAST CANCER CELLS (4T1)

Leila Rezakhani$_P$[1], Morteza Alizadeh[2], Akram Alizadeh[3]

[1] *Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran*

[2] *Department of tissue engineering, school of medicin,shahroud university of medical sciences,shahroud, Iran*

[3] *Department of Tissue Engineering and Applied Cell Sciences, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran*

Corresponding author's email: leila_rezakhani@yahoo.com

Objective: Due to the easy access and low cost, use of animal or plant exosomes in cancer treatment is promising. Fresh water crab had been used in traditional Iranian medicine to treat cancer. This study aimed to separate the exosome from the crab and investigate its anti-cancer properties on breast cancer cell line (4T1).

Materials and Methods: In this in vitro experimental study, crab hemolymph exosomes were isolated via precipitation method and characterized using electron microscopy, dynamic light scattering (DLS), and western blot. The protein concentration and total antioxidant capacity of exosomes were determined by bicinchoninic acid (BCA) and cupric reducing antioxidant capacity (CUPRAC). 4T1 cells and bone marrow mesenchymal stem cells (BMSCs) were treated with exosomes and the cell survival was assessed by RESAZURIN and MTT assays. Nitric oxide (NO) secretion from 4T1 cells, after treatment with exosomes was determined.

Results: Electron microscopy, DLS and western blotting for CD63, confirmed isolation of exosomes under the size range <100 nm. The total antioxidant capacity and protein concentration in exosomes were 1.003µM/ml and 650mg/ml respectively. RESAZURIN and MTT assays showed a decrease in cell survival of 4T1 ($p \leq 0.001$) after treatment with the exosomes. However, cell growth was observed in the exosomes treated BMSCs.

Conclusion: Crab hemolymph has protein-rich exosomes with antioxidant activity, therefore, it can have anti-cancer effects on 4T1 cells. These exosomes can be suggested for breast cancer therapeutics.

Keywords: Exosome, Breast Neoplasms, Fresh water crab, Antioxidant, Cell Survival

Abstract **#1074**

HARNESSING GLYCOSAMINOGLYCANS FOR DESIGNING IMMUNOMODULATORY NANOMEDICINE FOR DELIVERING SIRNA AND SMALL MOLECULE DRUGS

Oommen P. P Oommen[1], Sumanta Samanta[1], Oommen P Varghese[2], Vignesh K Rangasami[1]

[1] *Tampere University-Finland*

[2] *Uppsala University-Sweden*

Corresponding author's email: oommen.oommen@tuni.fi

Glycosaminoglycans are key components of the extracellular matrix that regulate several cellular processes such as cell signaling, proliferation, and migration. We harness the intrinsic biological properties of these polymers for engineering immunomodulatory nanomaterials and bulk hydrogels. We have recently discovered the unique property of hyaluronic acid (HA), to deliver siRNA across cellular barrier resulting in the development of the first biomimetic anionic transfection method for mammalian cells. We have also recently engineered HA-derived self-assembled nanocarriers by conjugating dexamethasone as the hydrophobic group and loaded the nanoparticles (NPs) with doxorubicin to obtain a size of 252 nm. These NPs specifically target the CD44 positive tumor cells and facilitated apoptosis. Interestingly, HA-NPs polarized the M2 macrophages to pro-inflammatory M1 phenotype with the upregulation of key cytokines thereby enhancing anti-tumor immunity. Finally, when we tested our HA-NPs in human whole blood, we found that HA-NPs mitigated the drug-mediated complement and coagulation cascade and suppressed platelet aggregation.

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Abstract **#1075**

INVESTIGATION OF UV CROSSLINKED KONJAC GLUCOMANNAN COMPOSITE FOR BIOMEDICAL ENGINEERING APPLICATIONS

Asdani Saifullah Dolbashid[1]

[1] *University of Malaya*

Corresponding author's email: asdanisaifullah@gmail.com

In this study, methyl acrylates (MA) and konjac glucomannan (KGM) were mixed together and UV-polymerized to obtain a layer of film that can be potentially used as artificial skin. UV polymerization provides high rates of polymerization thus allowing for a more efficient crosslinking of the monomers. For UV polymerization of the film, polyethylene glycol diacrylate (PEGDA) acted as crosslinker and Irgacure 2959 was used as photoinitiator in the experiment. All of the other components were kept at the same concentrations except for MA. The film was then left under UV light for 6-15 minutes for it to be polymerized.

The films were developed using three concentrations of MA (30%, 40%, and 50%). Degradation test, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), and contact angle measurement were used as parameters to determine the suitability of the film to be used as artificial skin. FTIR results showed both MA and KGM in the films, which proved the mixability and presence of both substances. Higher concentration of MA slows down degradation rate of the film, as the crosslink density of the polymers was higher. The wettability of the films also decreased with increased concentration of MA. Results from this experiment showed the effect of MA concentration on the composition and crosslink density of the films. The variation in concentration of MA used in this study had produced KGM-MA films with different characteristics which in turn will greatly helps in determining its potential use as artificial skin in the future.

Keywords

Artificial skin; Konjac Glucomannan; Biomaterial

Abstract **#1076**

RECELLULARIZATION OF DECELLULARIZED HUMAN FORESKIN IS ENHANCED BY SCAFFOLD PRETREATMENT WITH MONOPHOSPHORYL LIPID A :A NOVEL APPROACH FOR ENHANCED CELL MIGRATION

Shima Rahmatip[1,2], mehdi banitalebi-dehkordi[3], Ali jalili[4]

[1] *Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran*

[2] *Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran*

[3] *D Department of Molecular Medicine, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[4] *Cancer and Immunology Research Center , kurdistan university of medical sciences. Sanandaj, Iran*

Corresponding author's email: rahmati_may66@yahoo.com

Bioscaffolds and cells are two main components in the regeneration of damaged tissues via cell therapies. Migration and homing of cells are known as critical steps in this field. Umbilical cord stem cells are among the most well-known cell types for this purpose, with high benefits. The main objective of the present study was to evaluate the effect of the pretreatment of the foreskin acellular matrix (FAM) by monophosphoryl lipid A (MPLA) on the attraction of the human umbilical cord mesenchymal stem cells (hucMSC).

The expression of certain cell migration genes (VCAM-1, MMP-2) was studied using qRT-PCR. In addition to cell migration, transdifferentiation of these cells to the epidermal like cells were evaluated via immunohistochemistry of CK19 protein. The hucMSC showed more tissue tropism in the presence of MPLA pretreated FAM compared to the untreated control group. We confirmed this result by scanning electron microscopy (SEM). Furthermore, IHC data demonstrated that MPLA treatment increases the protein expression level of CK19.

In conclusion, pretreatment of acellular bioscaffolds by MPLA can increase the migration rate of cells and also transdifferentiation of hucMSC to epidermal like cells without growth factors. This strategy suggests a new approach in regenerative medicine

Abstract **#1077**

PREPARATION AND CHARACTERIZATION OF HIGHLY POROUS NANOCLAY-ENRICHED POLY (Ɛ-CAPROLACTONE) SCAFFOLDS AND EVALUATION OF OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

seyedeh elnaz enderamiₚ[1], seyedeh sara shafiei[1], mehdi shamsara[2], seyed ehsan enderami[3]

[1] Stem Cell and Regenerative Medicine,Institue of Medical Biotechnology, National Institue of Genetic Engineering & Biotechnology, Tehran, Iran

[2] National Research center for Transgenic Mouse, National Institue of Genetic Engineering & Biotechnology, Tehran, Iran

[3] Immunogenetics research center, Department of Medical Biotechnology, Faculty of Medicine, Mazandaran university of Medical Sciences, Sari, Iran

Corresponding author's email: aldin.enderami@gmail.com

In the last decades, tissue engineering has an effective role in introducing osteo bioimplants.1
Co-cell polymer with nano-clay can be one of the best implants for taking the place of bone failing and defecting in patients. 2,3
Human mesenchymal stem cells (hMSCs) and nano clay polymeric scaffolds can be biocompatible and biodegradable in the body.4
The nano clay_enriched polycaprolactone (PCL) scaffolds aid hMSCs proliferation and adhesion 5
Layered double hydroxide (LDH) nano clay can improve PCL properties in stem cells differentiation.3
Therefore the aim of this study is to fabricate bioactive, biointeractive highly porous(more than 94%) PCL scaffolds composited with different amounts of LDH at low temperatures.
In this study, highly porous PCL scaffold doped with different contents of LDH (ranging from (0.1 wt.% to 10 wt.%) simultaneously and the LDH attended smoothly dispersed throughout the PCL scaffold as confirmed by Energy dispersive X_ray analysis (EDX).
The viability, proliferation, morphology, attachment, and osteogenic differentiation of hMScs were assessed, and also mechanical behavior, degradation, and porosity of scaffold were examined. The osteo genes expression also approved osteogenic differentiation of hMSCs. These results demonstrated that the addition of LDH improved the osteogenic differentiation while compression strength, hydrophilicity, and degradation rate were also increased.

Keywords: layered double hydroxide, polycaprolactone, nano clay, bone tissue engineering

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Abstract **#1078**

PREPARATION OF BLACK PHOSPHORUS-LOADED GELATIN COMPOSITE POROUS SCAFFOLDS FOR PHOTOTHERMAL THERAPY AND TISSUE REGENERATION APPLICATIONS

Linawati Sutrisno$_P$[1,2], Huajian Chen[1,2], Naoki Kawazoe[1], Guoping Chen[1,2]

[1] *Research Center for Functional Materials, National Institute for Materials Science, Tsukuba, Japan.*

[2] *Department of Materials Science and Engineering, Graduate School of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Japan.*

Corresponding author's email: SUTRISNO.Linawati@nims.go.jp

Recently, surgical intervention has been the gold standard for breast tumor ablation, however, tumor recurrence and loss of adipose tissue decrease the progression-free survival (PFS) among patients. Photothermal therapy has been established as a safe modality of tumor ablation, while most of the photothermal agents are non-degradable, thus limiting its clinical application. Black phosphorus (BP) has attracted increasing attention due to its photothermal conversion efficiency, good biocompatibility and biodegradability. Due to the limited accumulation and easy diffusion of free nanoparticles, immobilization of nanoparticles into porous scaffolds for direct local implantation has become an attractive way for cancer cell ablation and simultaneous promotion of adipose tissue regeneration. In this study, composite porous scaffolds of gelatin and BP nanosheets were successfully prepared. The BP-gelatin composite scaffolds had a high photothermal ablation efficiency against MDA-MB231-Luc in vitro and in vivo. Furthermore, in vitro cell culture and in vivo implantation results revealed that the BP-gelatin composite scaffolds could up-regulate formation of lipid vacuoles and adipogenic-related gene expression for promoting adipogenic differentiation of hMSCs. Considering the ablation effect of tumor cells and promotive effect on adipogenic differentiation of hMSCs, the BP-gelatin composite porous scaffolds may offer a facile strategy to treat breast tumors.

Keywords

scaffold; tissue engineering; cancer therapy

Abstract **#1079**

BRAIN-ON-A-CHIP TECHNOLOGY FOR MIMICKING BRAIN PHYSIOLOGY AND NEUROPATHOLOGY

Hong Nam Kim$_P$[1,2]

[1] *Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea*

[2] *Division of Bi-Medical Science & Technology, KIST School, Korea University of Science and Technology (UST), Seoul, 02792, Republic of Korea*

Corresponding author's email: hongnam.kim@kist.re.kr

We present a brain-on-a-chip platform in which multiple types of human brain-originated brain cells were cocultured in a 3D microenvironment with perfusable brain microvasculature. The brain microvasculature was fabricated by using microneedles as templates. The removal of microneedles after the gelation of the collagen matrix allowed the formation of collagen channels. By seeding the brain microvascular endothelial cells in the luminal surface of the channel and embedding other neural cells in the collagen, a 3D brain model was prepared. The brain microvasculature physiology was confirmed through transendothelial permeability, immunofluorescence imaging, and cytokine assay. Neuropathology such as brain tumor and infectious diseases were recapitulated in the brain-on-a-chip platform and pathophysiological features, as well as the pharmaceutical candidates, were evaluated in the platforms. We believe that the brain-on-a-chip technology can not only help the unraveling of underlying mechanisms of brain diseases but also testing the efficacy of potential pharmaceutic candidates.

Abstract **#1080**

ANTIOXIDANT PHAGE NANOFIBERS INDUCING ANGIOGENESIS

So Young Yoo[P1], Su-Nam Jeong[1]
[1] Pusan National University
Corresponding author's email: yoosy2@gmail.com

Instructive biomaterials provide a vascular niche and protect oxidative stress in injured tissue. Herein, we demonstrated that Arg-Gly- Asp (RGD)-engineered bacteriophage nanofibers, furtherly modified with antioxidant/anti-inflammatory peptides, provide a vascular and anti-oxidant niche, thereby having cytoprotective functions against cellular oxidative stress. These bioinspired RGD-engineered bacteriophage nanofibers can serve as a novel therpeutic platform for ischemic diseases.
Keywords: antioxidant phage nanofiber, stem cells, ischemic diseases

References

Biomacromolecules. 20, 3658, 2019.

Abstract **#1081**

PHAGE BASED DE NOVO VASCULAR NICHE ENGINEERING

So Young Yoo[P1], Kshitiz Raj Shrestha[1]

[1] Pusan National University

Corresponding author's email: yoosy2@gmail.com

Implanted stem cell survival/proliferation is the major challenge in stem cell therapy for ischemic disease treatment. Extracellular niche plays a crucial role in overcoming surrounding harsh pathological environments as well as in stem cell differentiation and other important functions. However, an artificial niche achieved so far is not successful especially to fulfill the soft tissue niche. Herein, we exploited an engineered phage displaying cell adhesive peptides with a suitable structural cue can provide a controlled soft tissue niche to increase the angiogenic efficacy of endothelial progenitor cell (EPC) and to improve the survival of the implanted EPC at the ischemic site. Engineered phage successfully modulates physical and biochemical cues to achieve an appropriate niche in the functioning of implanted EPC, thus providing a convenient vascular soft tissue niche. These biomimetic phages may serve as a promising therapeutic option for curing ischemic diseases.

Keywords: phage, stem cells, ischemic diseases, vascular niche

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Abstract **#1082**

NOVEL ONCOLYTIC VIRUS ARMED WITH CANCER SUICIDE GENE AND NORMAL VASCULOGENIC GENE

So Young Yoo$_P$[1], Su-Nam Jeong[1]

[1] *Pusan National University*

Corresponding author's email: yoosy2@gmail.com

Here, we developed a novel oncolytic vaccinia virus (NOV) with the dual advantages of cancer selectivity and normal vessel reconstructive activity by replacing the viral thymidine kinase (vTk) and vaccinia growth factor (VGF) genes with genes encoding TNF-related apoptosis-inducing ligand (TRAIL) and angiopoietin 1 (Ang1), respectively. The pan-cancer-specific oncolytic potency of NOV was confirmed in various human and mouse cancer cell lines (colon, liver, pancreas, cholangiocarcinoma, cervical cancer, osteosarcoma, and melanoma). Vaccinia virus (VV) treatment directly induced early apoptosis in tumors within 24 h, and this effect was enhanced with further engineering; VGF and Tk deletion with Ang1 and TRAIL insertion. Meanwhile, treatment with the conventional anti-cancer drug cisplatin did not induce apoptosis. A virus-treated CT26 mouse colon cancer syngeneic model showed attenuated tumor growth, which was in accordance with the results of percent survival measurement, CD8 expression analysis, and TUNEL staining with advanced genetic engineering (vAng1 < vTRAIL < NOV). Taken together, our results indicate that NOV induces cancer tissue apoptosis and anti-tumor immunity and may constitute a highly advantageous therapeutic agent for next-generation solid tumor virotherapy with pan-cancer-specific oncolytic activity and high biosafety.

Keywords: TRAIL; angiopoietin 1; oncolytic virus

References

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Abstract **#1083**

FUNCTIONAL AND BIOCOMPATIBILITY OF ANTIMICROBIAL BIOCOMPOSITE MEMBRANE IN ORTHOPEDICS APPLICATION: THE PRELIMINARY OUTCOME

Sarah Haryati Mohd Zohari[P][1,2,3,4,5,6,7,8]

[1] *Prof Dr Ahmad Hafiz Zulkifly*

[2] *Dr Nurul Hafiza Mohd Jan*

[3] *Rosnani Abd Jalil*

[4] *Mohd Zulfadzli Ibrahim*

[5] *Zahana Abdul Hamid*

[6] *Muhamad Ezham Zainal Abdullah*

[7] *Che Nor Zarida Che Seman*

[8] *Asst Prof Dr Zunariah Buyong*

Corresponding author's email: saraharyati9893@gmail.com

There a lot of membranes types have been used in Guided Bone Regeneration (GBR) surgery procedure. The issue of using the membrane implantation are it can cause infection and contributes to the cause for the procedure failure. In order to prevent bacterial colonisation, multiple antibiotics have been used. Nevertheless, it contributes to the side-effects and bacterial resistance. Hence, the idea of triple layered and functionally graded antimicrobial biocomposite membrane comprising of poly (lactic-co-glycolic acid) (PLGA) matrix with bioactive apatite filling and lauric acid (LA) has been proposed as a potential to provide a solution to this problem. Using the Critical Size Defect (CSD) technique in the New Zealand White Rabbit, this study seeks to investigate the biocompatibility and potential effect of the antimicrobial bio composite membrane through in vivo analysis. Eight New Zealand White Rabbits were used in this study. A CSD of approximately was surgically drilled at the proximal tibial metaphysis, approximately 1 cm from the knee joint. 1 cm x 2 cm of the bio composite antimicrobial membrane was inserted at the tibia site. The rabbits were euthanized then the bone samples were taken for further analysis. Gross observation conducted showed a very good contact between the bone and implant membrane for all rabbit models. Bone cortical rim is visible in the radiographic images assessment. Histological analysis indicates that a section of material in close contact with the new bone. Close-up view of new bone shows the presence of osteoid with adjacent osteoblasts.

Abstract **#1084**

SPECIFIC SUGAR TARGETING BIFUNCTIONAL NANOPARTICLES FOR THE DETECTION OF EXOSOMES DERIVED FROM PANCREATIC CANCER PATIENTS

Yonghyun Choi[P][1], Uiseon Park[2], Hyung-Jun Koo[3], Jin-seok Park[4], Don Haeng Lee[4], Kyobum Kim[5], Jonghoon Choi[1]

[1] *School of Integrative Engineering, Chung-Ang University, Seoul, Republic of Korea*

[2] *Department of Bioengineering and Nano-Bioengineering, Incheon National University, Incheon, 22012, Republic of Korea*

[3] *Department of Chemical and Biomolecular Engineering, Seoul National University of Science and Technology, Seoul, 01811, Republic of Korea*

[4] *Department of Internal Medicine, Inha University School of Medicine, Incheon, 22212, Republic of Korea*

[5] *Department of Chemical & Biochemical Engineering, Dongguk University, Seoul, 04620, Republic of Korea*

Corresponding author's email: dydgus5057@gmail.com

The mortality rate for pancreatic cancer is high due to lack of powerful early diagnosis technologies. Main obstacles for improving clinical trial result are a diagnosis at an early stage and limited therapeutic efficacy. To overcoming this problem, recently tumor-derived exosomes are attracting interest in cancer diagnosis and treatment field. Membranous vesicles that may include novel markers same as proteins, RNAs and DNA, can be found in the circulating blood. Here we demonstrate recent technologies highlighting the cancer exosome detection by introducing lectin and Janus nanoparticles. Lectins are glycoproteins that exhibit specific binding affinity for the saccharide moiety of glycoproteins. Cancer cells are known to express or secrete glycoproteins with specific saccharide chain like sialic acid in pancreatic cancer. Therefore, in this study, we introduce the pancreatic cancer cell-derived exosome detection technology based on the specific binding of lectins to the characteristic glycan profiles on the surface of exosomes for the first time. Lectins with a high and specific affinity to sialic acid or fucose were attached on the bifunctional nanoparticles (i.e., Janus nanoparticles) to interact with pancreatic cancer cell-derived exosomes in a microfluidic device. Pancreatic cancer cell-derived exosomes from both cell-lines and patient's plasma samples were successfully captured on the lectin-conjugated Janus. In addition, exosome detection using our platform was able to differentiate between metastatic and non-metastatic pancreatic cancer cells. This study opens the possibility to achieve a new early diagnosis marker based on the glycan properties of pancreatic cancer-derived exosomes.

Keywords

Exosome; Pancreatic cancer; Lectin

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Abstract **#1085**

SPATIALLY SEPARATED BIOACTIVATION OF ENCAPSULATION MEMBRANES REDUCES FIBROSIS AND ENHANCES ISLET CELL SURVIVAL

Richard Tan[P][1,2], Nicole Hallahan[1,2], Elena Kosobrodova[3], Marcela Bilek[3], Peter Thorn[1,2], Steven Wise[1,2]

[1] *Faculty of Medicine and Health, University of Sydney*

[2] *Charles Perkins Centre, University of Sydney*

[3] *School of Physics, University of Sydney*

Corresponding author's email: richard.tan@sydney.edu.au

Encapsulation devices are an emerging technology designed to prevent the immunorejection of replacement cells in regenerative therapies. However, traditional polymers used in current devices are poor substrates for cell attachment and induce fibrosis upon implantation, impacting long-term therapeutic cell viability. Bioactivation of polymer surfaces improves local host responses to materials and here we make the first steps towards demonstrating the utility of this approach to improve cell survival within encapsulation implants. Using therapeutic islet cells as an exemplar cell therapy, we show that internal surface coatings improve islet cell attachment and viability while distinct external coatings modulate local foreign body responses. Using plasma surface functionalisation (PIII), we employ hollow semi-porous polyethersulfone (PES) fibres and coat the internal surfaces with the extracellular matrix protein fibronectin (FN) to enhance islet cell attachment. Separately, the external fibre surface is coated with the anti-inflammatory cytokine interleukin-4 (IL-4) to polarise local macrophages to an M2 (anti-inflammatory) phenotype, muting the fibrotic response. Bioluminescent murine islet cells were then loaded into dual FN/IL-4 coated fibers and evaluated in a mouseback model for 14 days. Dual FN/IL-4 fibers showed striking reductions in immune cell accumulation and elevated levels of the M2 macrophage phenotype, consistent with suppression of fibrotic encapsulation and enhanced angiogenesis. These changes led to markedly enhanced islet cell survival and importantly to functional integration of the implant with the host vasculature. Dual FN/IL-4 surface coatings drive multi-faceted improvements in islet cell survival and function, with significant implications for improving clinical translation of therapeutic macroencapsulation devices.

Abstract **#1086**

DEVELOPMENTS OF BIOFABRICATED STRUCTURES IN NOVEL TREATMENTS FOR LOWER URINARY TRACT SYMPTOMS

Tetsuya Imamura$_P$[1]

[1] *Shinshu University School of Medicine*

Corresponding author's email: imatetu@shinshu-u.ac.jp

We have vigorously investigated treatments of lower urinary tract symptoms (LUTS) with “next generation tissue engineering”, which are composed of three concepts, conventional tissue engineering-based methodology, construction of suitable technology according to microenvironment, and incorporation of novel biotechnology. We showed that bone marrow- or adipose-derived cells these cells had the potential to reconstruct functional urinary bladders with frozen- and/or radiation-injury models. The cells were delivered into recipient bladders by using two methods, direct-injection of single cells and patch-transplantation of cell sheets produced in temperature-responsive culture dishes. Both methods successfully reconstructed bladder tissues and restored the functions. However, there are some limitations for each method. In the direct-injection method, survival rates of the implanted cells were very low within the recipient tissues. For the cell sheet patch-transplantation method, the sheets were too thin and weak to be reliably delivered to the recipient tissues. To overcome these limitations, we have attempted to biofabricate structures with a three-dimensional (3D) bioprinting robot system. Previously, we transplanted the biofabricate structures, which were composed of bone marrow-derived cells, into the irradiation-injured rat urinary bladders(1). We showed that the structures survived, and blood vessels invaded them from the adjacent recipient tissues. The bone marrow-derived cells constructing the structures differentiated into smooth muscle cells and formed smooth muscle clusters. In addition, the structure-transplanted rats had improved urinary frequency symptoms and decreased residual volume. In this symposium, we would like to indicate that the biofabricated structures have great potentials to treat patients with LUTS.

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Abstract **#1087**

MORPHOLOGY-BASED EVALUATION FOR DRUG RESPONSES IN HETEROGENEOUS NEURAL CELL POPULATION

Yuta Imai[P][1], Kei Kanie[1,2], Madoka Iida[3], Masahisa Katsuno[3], Ryuji Kato[1,2]

[1] *Grad. Sch. Pharm. Sci., Nagoya Univ.*

[2] *Inst. Nano-Life-Systems, Nagoya Univ.*

[3] *Grad. Sch. Med., Nagoya Univ.*

Corresponding author's email: imai.yuuta@a.mbox.nagoya-u.ac.jp

In recent years, cell-based assays have been attracting attention due to the growing expectations for animal replacement experiments. The development of a drug Responses evaluation system for neural cells has been progressing toward the treatment of neurodegenerative diseases using such techniques.

Human neural cells are highly heterogeneous within a population, resulting in various, sometimes non-reproducible, drug response as a bulk population. Therefore, it has been difficult to evaluate drug sensitivity using the averaged values from bulk cells. To breakthrough such difficulty, it is expected to establish an evaluation system that can sensitively focus on the rare population cells that reacts to the drug, and objectively and quantitatively extract their reaction within the heterogeneous population.

Our research group has been developing morphology-based drug response profiling by combining image processing and machine learning technologies [1,2]. In this analysis, all the individual cells are quantitated through the live time-course phase contrast images and total population data were numerically accumulated in the database. We developed a new algorithm to focus on the “rare population of cells that shows great morphological changes”, designated as “in silico FOCUS”. By this new analysis method, we show that the expected responses of neural model cells to the drug can be sensitively and quantitatively predicted. This method can contribute to new morphological analysis that can quantify rare populational reactions in bulk cells.

Keywords

Image analysis; Neural cell; Drug screening

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Abstract #1088

ONE-STEP APPROACH OF THERMAL RESPONSIVE GENIPIN-CROSSLINKED GELATIN HYDROGEL FOR FULL-THICKNESS SKIN LOSS

Nike Dewi Utami[P][1], Mh Busra Fauzi[1], Haliza Katas[2], Ruszymah Hj Idrus[1]

[1] *Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia*

2 Centre for Drug Delivery Research, Faculty of Pharmacy, Universiti Kebangsaan Malaysia

Corresponding author's email: nike.dewiutami@gmail.com

Gelatin has been widely used in hydrogel form for drug delivery and as cutaneous template for skin wound applications. Unfortunately, gelatin hydrogel (GH) has low mechanical strength and thermally unstable at body temperature of 37°C. Therefore, a crosslinking agent is required to increase the mechanical properties and thermal stability of gelatin hydrogel. This study was conducted to characterize the mechanical and thermal properties of polymerized gelatin hydrogel crosslinked with genipin (GNP). The fabricated hydrogel was tested by texture analyzer, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The GNP-crosslinked GH (GNP_GH) scaffolds were synthesized by mixing different ratios of gelatin (w/v; 5%, 10% and 15%) and GNP (w/v; final concentration of 0.1% and 0.5%) to achieve gelation time within 3 minutes. The results revealed that 0.1%GNP_GH 10%, 0.1%GNP_GH15% and 0.5%GNP_GH15% turned to hydrogel form within 3 minutes at 22ºC. All groups exhibited higher stiffness, resilience, adhesive force and adhesiveness compared to the non-crosslinked. The DSC and TGA thermogram shown that genipin crosslinking successfully enhance the thermal stability of gelatin bioscaffolds. These results demonstrated that gelatin hydrogel could be used as acellular treatment for full-thickness skin loss.

Abstract **#1089**

IN SITU INTERVERTEBRAL DISC REGENERATION USING PEPTIDE FUNCTIONALIZED CELLULOSE-ALGINATE BASED DOUBLE NETWORK HYDROGEL

Chia-Yu Ho[P][1], Tzu-Wei Wang[1]

[1] *Department of Materials Science and Engineering, National Tsing Hua University*

Corresponding author's email: j0617johnny@gmail.com

Intervertebral disk diseases (IDD) describe the breakdown of intervertebral disc (IVD) of the spine. Tissue engineering has provided an alternative therapeutic possibility for degenerative disc diseases. Currently, clinical treatments have limitations that cannot fully reconstruct the hierarchical structure and mimic the function of native intervertebral disc (IVD). In this study, we develop an IVD scaffold based on cellulose and alginate. A total IVD is created with the combination of cellulose-based nucleus pulposus (NP) and annulus fibrosus (AF) composed of cellulose-alginate double network for mimicking native IVD tissue. Moreover, mesenchymal stem cell (MSC) homing peptide and RGD cell adhesion peptide are modified on the NP part and AF part of the IVD scaffold, respectively, to promote endogenous stem cells recruitment and enhance cell adhesion as well as proliferation. Additionally, with the addition of growth and differentiation factor-5 (GDF-5), the recruiting MSCs can differentiate into an NP-like phenotype. The mechanical property of the reported hydrogel was enhanced via the addition of alginate network and drying in confined condition method. The grafting ratio of functional peptide motifs was further determined by NMR and FTIR spectra. The present work suggests that the reported double network hydrogel may serve as a promising candidate for the applications in IVD regeneration as well as for other biomedical applications in tissue engineering.

Keywords

double network hydrogel; functional peptide motif; intervertebral disc regeneration

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Abstract **#1091**

EFFECT OF KIDNEY STEM CELLS ON INFLAMMATORY FACTORS IN RATS WITH DIABETIC NEPHROPATHY

Zeinab Rafiee$_P$[1], Mahmoud Orazizadeh[2], Esrafil Mansouri[3]

[1] *Cellular and molecular research center, Ph.D student of Anatomical sciences,Ahvaz Jundishapur University of Medical Sciences,Ahvaz,Iran*

[2] *Cellular and molecular research center,Department of Anatomical sciences,Faculty of medicine,Ahvaz Jundishapur University of Medical Sciences,Ahvaz,Iran*

[3] *Cellular and molecular research center,Department of Anatomical sciences,Faculty of medicine,Ahvaz Jundishapur University of Medical Sciences,Ahvaz, Iran*

Corresponding author's email: zeinabrafiee@yahoo.com

Mesenchymal stem cells (MSCs) were proposed as a critical therapeutic candidate in diabetic nephropathy (DN). Renal stem cells as a source for repairing are controversial. The purpose of the present study was to evaluate the effect of kidney rat stem cells on DN. After evaluation of surface stem cell markers by flow cytometry analysis, MSCs that were labeled with CM-Dil were injected to experimental groups via tail vein (2×106cells/rat). To establish the diabetic nephropathy rat model STZ(60mg/kg) was used. The IL1-β, TGF-β, miRNA 192, miRNA29a gene expression were assessed three weeks after induction of early diabetic nephropathy by using quantitative Real-time PCR. The renal stem cells 24 h after labelling with CM-Dil fluorescent dye were detected in the renal tissues. miRNA192, TGF-β and IL1-β Genes expression upregulated in diabetic group. The expression of miRNA29a significantly down-regulated 21 days after injection of STZ. H and E staining showed in the presence of adult kidney stem cells histopathological changes were improved. The protective effect of MSCs was closely related to the inactivation inflammatory factors. Therefore, the MSCs may be a promising novel treatment for early DN.

Keywords

stem cell therapy; inflammatory cytokines; Diabetic Nephropathy

Abstract **#1092**

FABRICATION OF A MIMETIC VASCULAR GRAFT USING A FOUR-AXIS FUSED DEPOSITIONING SYSTEM.

Kenny van Kampen$_P$[1], Elena Olaret[2], Izabela-Christina Stancu[2], Carlos Mota[1], Lorenzo Moroni[1]

[1] *Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University*

[2] *Advanced Polymer Materials Group, University Politehnica of Bucharest*

Corresponding author's email: k.vankampen@maastrichtuniversity.nl

Vascular grafts with long-term patency are in great clinical demand. With current limitations in the use of autologous grafts, biofabrication offers a suitable alternative. Specifically a four-axis fused deposition modeling (FDM) system has the ability to create tubular constructs with a broad range of mechanical properties. The formation of a tight endothelial barrier and having a surrounding smooth muscle cell layer is vital for vascular graft patency. Here we used a four-axis FDM system to create a vascular construct with matching mechanical properties that supports the formation of an endothelial monolayer in the lumen and surrounding smooth muscle cell layer.

A 2 mm diameter mandrel was attached to a DC-motor with a rotational speed of 3000 RPM. Polycaprolactone was molten in a syringe and dispensed on the mandrel while it was rotating. After deposition of the thin fibres, the mandrel with the fibers was transferred to a custom CNC-machine with a controllable fourth rotary axis where a script was generated to create the pathway for the supporting fibers. Scaffolds were disinfected with 70% ethanol before a combination of smooth muscle cells (A10) and endothelial cells (HUVEC) were seeded on the outside and the lumen of the scaffold respectively.

During this study, a four axis fiber extrusion technique was used to fabricate a vascular scaffold with defined mechanical properties. In addition endothelial cells were able to form a monolayer in the lumen of the scaffold while smooth muscle cells migrated from the outside of the scaffold towards the lumen.

Keywords

Biofabrication; Biomimicry; Vascular graft

Abstract **#1093**

EXPIRED PLATELET CONCENTRATES AS A SOURCE OF HUMAN PLATELET LYSATE FOR XENOGENEIC-FREE EXPANSION OF PRIMARY HUMAN CELLS

Jia Xian Law[P][1], Muhd Najib Fathi bin Hassan[1], Yee Loong Tang[1], Min Hwei Ng[1]

[1] *Universiti Kebangsaan Malaysia*

Corresponding author's email: lawjx@ppukm.ukm.edu.my

Foetal bovine serum (FBS) has been used for in vitro expansion of primary human cells for decades. However, the use of FBS is controversial as it has been linked with safety issues related to the contamination of cultured cells with animal proteins and risk of zoonotic pathogen transmission. In this study, we examined the potential of using human platelet lysate (HPL) prepared from expired human platelet concentrates for the xenogeneic expansion of primary human cells, i.e. Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs), dermal fibroblasts (DF) and chondrocytes. From the results, we found that HPL was superior compared to the FBS in promoting the WJ-MSC, DF and chondrocyte proliferation whereby the population doubling time was significantly shorter. In addition, we also observed that WJ-MSCs, DF and chondrocytes cultured with HPL were smaller in size compared those expanded with FBS. As a result, significantly higher cell yield was obtained with HPL compared to FBS. Nonetheless, just like FBS, HPL cannot prevent dedifferentiation of chondrocytes. As for fibroblasts, we observed that HPL reduced the extracellular matrix gene expression and slowed down the cell migration. In conclusion, HPL can be used for the expansion of primary cells especially those expanded for clinical use.

Keywords

Human platelet lysate; Cell culture

Abstract **#1094**

OVINE COLLAGEN TYPE I BIOMATRIX INTEGRATED WITH ANTIBACTERIAL PROPERTIES VIA PLASMA POLYMERISATION AS ACELLULAR SKIN SUBSTITUTE FOR CHRONIC SKIN INJURY

Nor Amirrah Ibrahim[P][1], Fauzi Mh Busra[1], Ruzymah Hj Idrus[1,2], Mohd Farhanulhakim Razip Wee[3], Tabata Yasuhiko[4]

[1] *Centre of Tissue Engineering and Regenerative Medicine, Universiti Kebangsaan Malaysia*

[2] *Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia*

[3] *Institute of Microengineering and Nanoelectronics, Universiti Kebangsaan Malaysia*

[4] *Laboratory of Biomaterials, Department of Regeneration Science and Engineering, Institute for Frontier Life and Medical Sciences, Kyoto University*

Corresponding author's email: noramirrahibrahim@gmail.com

Optimal wound dressings for skin wounds can mimic the native extracellular matrix and act as a barrier between the wound bed and the external environment. Infection from chronic wounds can delay progression and lead to debilitation. This study aimed to evaluate the antibacterial essential oil (R)- (-)-Carvone coating of a monolayer ovine collagen type I (OCT-I) biomatrix. Genipin (GNP) and dehydrothermal treatment (DHT) were used to crosslink OTC-I scaffold to improve its thermal and enzyme stability. Carvone has antimicrobial properties, and its functional groups are deposited to coat lyophilised porous OTC-I scaffold through plasma polymerisation treatment (OTC-I_ppCar). The physicochemical, biodegradation, biomechanical, biocompatibility, and antibacterial properties for composite biomatrix were evaluated. The results revealed that GNP crosslinked OTC-I scaffold (OTC-I_GNP) showed better physicochemical and mechanical properties compared to DHT crosslinked OTC-I scaffold (OTC-I_DHT) and non-crosslinked OTC-I scaffold (OTC-I_NC). Both composited biomatrix with Carvone deposition OTC-I_GNP_ppCar and OTC-I_DHT_ppCar demonstrated antibacterial properties and are compatible with human dermal skin fibroblasts (HDF) as it showed cell attachment and proliferation. The composite biomatrix shows potential for management of future treatment of chronic wounds such as diabetic ulcers.

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Abstract **#1095**

MICROFLOW REACTOR HYPHENATED WITH ION MOBILITY-MASS SPECTROMETRY FOR DRUG DISCOVERY

Darya Hadavi[1], Peiliang Han[1], Maarten Honing[1]

[1] *Maastricht MultiModal Molecular Imaging Institute (M4I), Maastricht University, The Netherlands*

Corresponding author's email: d.hadavi@maastrichtuniversity.nl

Continuous flow chemistry is an efficient, sustainable and green approach for chemical synthesis that surpasses some of the limitations of the traditional batch chemistry. Along with the multiple advantages of a flow reactor, it could be directly connected to the analytical techniques for on-line monitoring of a chemical reaction and ensure the quality by design. Here, we aim to use ion mobility, mass and tandem mass spectrometry (IMS-MS and MS/MS) for the on-line analysis of a pharmaceutically relevant chemical reaction. We carried out a model hetero-Diels Alder reaction in a microflow reactor directly connected to the IMS-MS and MS/MS using either electrospray or atmospheric pressure photo ionization methods. We were able to monitor the reaction mechanism of the Diels Alder reaction and structurally characterize the reaction product and synthesis side-products. The chosen approach enabled identification of two isomers of the main reaction product. A new strategy to annotate the ion mobility spectrum in the absence of standard molecules was introduced and tested for its validity. This was achieved by determining the survival yield of each isomer upon ion mobility separation and density functional theory calculations. This approach was verified by comparing the theoretically driven collision cross section values to the experimental data. In this paper, we demonstrated the potential of combined IMS-MS and MS/MS on-line analysis platform to investigate, monitor and characterize structural isomers in the millisecond time scale.

Abstract **#1096**

4D-BIOPRINTED INTERVERTEBRAL DISC AND FUNCTIONAL GRADIENTS

Catherine Le Visage$_P$[1], Perrine de Villemagne[2], Benoit Rosa[2], Paul Patreau[2], Vianney Delplace[1], Pierre Weiss[1], Franck Halary[3], Jerome Guicheux[1], Jean-Yves Hascoët[2]

[1] *Inserm U1229 RMeS Regenerative Medicine and Skeleton, Nantes, France*

[2] *Institut de Recherche en Génie Civil et Mécanique (GeM), CNRS 6183, Centrale Nantes, France*

[3] *Centre de Recherche en Transplantation et Immunologie (CRTI), INSERM U1064, Nantes, France*

Corresponding author's email: catherine.levisage@inserm.fr

Our ability to create physiological tissues with complex architectures, including spatial distribution of cells and extracellular matrix components, remains a challenge. The intervertebral disc (IVD), a fibro-cartilaginous structure acting as a natural shock absorber, contains an outer network of collagen fibers (Annulus fibrosus, AF) surrounding a central gelatinous (Nucleus pulposus, NP). Current approaches to engineer a functional IVD model, as an alternative to animal experiments, fail to recapitulate the physiological AF-NP gradient.

In this context, we have developed an in-house extrusion bioprinter with temperature controllers for both the printing stage and dispensing syringes and a pneumatic control allowing high spatial displacement resolution (0.1µm). A gelatin-alginate (7%:1%) bioink was optimized with the addition of hyaluronic acid (1%) and RGD adhesion groups to promote cell proliferation. The printability and long-term stability of bioprinted constructs were assessed by generating 1x1x0.5 cm3 cubes with high shape fidelity. Disc cells were harvested from young sheep (n=6) and were used to evaluate the bioink cytocompatibility. Disc cells were bioprinted and cell viability (Live&Dead), cell shape (actin immunostaining) and proliferation (EdU staining) were assessed after 1 month. Over 90% of cells were viable, proliferation was evidenced, with elongated cells observed within the constructs. Finally, MRI data from sheep IVDs were used to generate a full IVD model. Current efforts are focused on a mixing nozzle to generate the AF-NP gradient. Bioinks will be printed according to pre-defined trajectories built upon a gradient analysis of the distribution of collagen type II/type I on immunohistochemical sections of sheep IVDs.

Abstract #1097

MODEL-BASED CULTURE OPTIMISATION BASED ON SINGLE-CELL QUANTITATIVE CONFOCAL IMAGE ANALYSIS

Mark Allenby$_P$[1], Athanasios Mantalaris[2], Maria Woodruff[3]

[1] *University of Queensland (UQ)*

2 Georgia Institute of Technology

3 Queensland University of Technology (QUT)

Corresponding author's email: m.allenby@uq.edu.au

Tissue engineering has long championed the importance of 3D culture models which mimic physiological form and function. While 3D cultures platforms can achieve higher product quality and quantity, few 3D platforms have been translated from the research bench, and industrial cell and tissue biomanufacturers remain smitten with stirred tank reactors due to ease of monitoring, reproducibility, and scalability. Here we illustrate recent work leveraging image-based mathematical models of cell culture to design and engineer better culture platforms. Platforms include marrow-mimicry hollow fibre perfusion reactors to biomanufacture red blood cells [1], patient-specific 3D printed bone reactors to optimise medium transport at scale [2], and melt-electrowritten microscaffolds to optimise pore bridging kinetics [3], among others. In all cases, custom-programmed confocal image analyses captured hundreds to millions of individual cell positions, shapes, and types to understand the spatial distribution of tissue growth. These large imaging datasets, with additional culture assays, were leveraged to parameterise mathematical models of scaffold tissue growth. This approach was able to enhance culture design through predictive modelling. Specific 3D printed scaffolds pore sizes and shapes could be selected for rapid tissue proliferation or desired morphology. Bioreactor medium composition and perfusion could be tailored to leverage autonomous cytokine release and minimize supplemented biofactor expense. Altogether, quantitative confocal image analysis offers a methodology to better monitor 3D culture kinetics to improve biomanufacturing process reproducibility and understand challenges in scalability.

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Abstract #1098

AUTOLOGOUS CELL-BASED VASCULAR GRAFT USING KENZAN METHOD BIO-3D PRINTER FOR CLINICAL APPLICATION.

Manabu Itoh$_P$[1]

[1] *Department of Thoracic and Cardiovascular Surgery Saga University Faculty of Medicine*

Corresponding author's email: itomana@cc.saga-u.ac.jp

Conventional small caliber synthetic vascular graft with a diameter of less than 6 mm made of foreign substances such as PTFE (polytetrafluoroethylene) have many problems to be solved in terms of antithrombogenicity, anti-infectivity, biocompatibility, etc. Stem cell technology and tissue engineering technology have remarkably developed in recent years. Especially, 3D bioprinting is an emerging and rapidly expanding field with many exciting applications. We used a “Kenzan method Bio-3D Printer” to assemble multicellular spheroids to construct a three-dimensional structure predesigned on a computer system. This technique has enabled the production of tubular tissues only containing cells, without any exogenous scaffolds. Currently, we have started clinical research of autologous cell-based artificial blood vessels made with this Bio-3D printer as arteriovenous shunt used for hemodialysis. In this symposium, we will introduce the current situation, challenges, and future perspective of the clinical development of artificial blood vessels made of cells of small caliber using Kenzan method Bio-3D printers.

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Abstract **#1099**

CONTROLLED RELEASE OF BONE MORPHOGENETIC PROTEIN-2 FROM MAGNESIUM HYDROXIDE-INCORPORATED PLGA COMPOSITE FOR BONE REGENERATION

Dong Keun Han[P][1], Da-Seul Kim[1,2], Tarek M. Bedair[1], Chang Kyu Lee[3], In Bo Han[4], Tae-Hyung Kim[2]

[1] *CHA university*

[2] *Chung-Ang university*

[3] *Keimyung University Dongsan Medical Center*

[4] *CHA University*

Corresponding author's email: dkhan@cha.ac.kr

Spinal fusion surgery connects two or more vertebrae to stabilize a damaged spine; however, the rate of failure of fusion (Pseudarthrosis) is still high. The bone morphogenetic protein-2 (BMP2) has been approved to minimize pseudarthrosis for use in humans. Here in, we developed a poly(lactide-co-glycolide) (PLGA) composite incorporated with magnesium hydroxide (MH) nanoparticles for the delivery of BMP2. This study was evaluated the effects of released BMP2 from BMP2-immobilized PLGA/MH composite scaffold in an in vitro analysis and an in vivo mice spinal fusion model. The PLGA/MH composite films were fabricated via solvent casting technique. The surface of the PLGA/MH composite scaffold was modified with polydopamine (PDA) to effectively immobilize BMP2 on the PLGA/MH composite scaffold. Analysis of the scaffold revealed that PDA modified PLGA/MH composite improved hydrophilicity, degradation performance, neutralization effects, and increased BMP2 loading efficiency. Additionally, releasing BMP2 from the PLGA/MH scaffold significantly promoted the proliferation and osteogenic differentiation of MC3T3-E1 cells. The pH neutralization effect significantly increased in MC3T3-E1 cells cultured on the BMP2-immobilized PLGA/MH scaffold. In our animal study, the BMP2-immobilized PLGA/MH scaffold significantly enhanced bone regeneration by increasing osteogenesis and suppressing inflammatory responses. These results collectively demonstrate that the BMP2-immobilized PLGA/MH scaffold offers great potential in effectively inducing bone formation as a bioinspired material for spinal fusion surgery.

Keywords

Spinal fusion; Bone regeneration; Bone morphogenetic protein-2

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Abstract **#1100**

PLASMA-MODIFIED PIEZOELECTRIC NANOFIBROUS CONDUITS FOR NERVE TISSUE ENGINEERING APPLICATIONS

Mahtab Asadian$_P$[1,2], Iullia Onyshchenko[1], Alexander Van Tongel[3], Rino Morent[1], Nathalie De Geyter[1]

[1] *Research Unit Plasma Technology (RUPT), Department of Applied Physics, Ghent University*

[2] *Prometheus Division of Skeletal Tissue Engineering, Department of Materials Science, KU Leuven University*

[3] *Orthopedic Surgery and Traumatology, Department of Human Structure and Repair, Ghent University*

Corresponding author's email: Mahtab.asadian@UGent.be

Recently, electrospun conduits have attracted considerable attention for nerve regeneration owing to their similarity to the nerve-extracellular matrix. To precisely mimic the nerve structure, these constructs should provide a challenging structure: random nanofibers in the outer layer and aligned ones on the inner side. It has been stated that piezoelectric materials can actively stimulate nerve regeneration as they create transient charges in response to mechanical strain. In addition, surface chemical properties play a leading role in cell-biomaterials interactions. Non-thermal plasma treatment is a well-known technique to change the surface chemical properties of the material, however, the incorporation of different functional groups by this method on piezoelectric nerve conduits is poorly investigated in the literature.

Therefore, in this research, polyvinylidene fluoride trifluoroethylene (PVDF-TrFe) conduits were fabricated by a novel two-pole structure electrospinning collector after which they were exposed to an atmospheric pressure plasma jet. The morphology, mechanical properties, wettability, chemical characteristics and cell affinity of the conduits before and after plasma treatment were assessed comprehensively.

The obtained images by scanning electron microscopy demonstrated that the designed collector is successful in obtaining the required nanofibers arrangement on the conduit walls. Furthermore, the hydrophilicity of the PVDF-TrFe conduits can be significantly increased due to the incorporation of polar oxygen-containing groups after plasma modification. In addition, it was also observed that cell-material interactions were improved due to applied plasma treatment. As such, the combination of using the fabricated piezoelectric nanofibrous conduits with plasma modification ensures a bright future for these constructs in nerve regeneration.

Keywords

Nerve tissue engineering; Nanofibrous conduit ; Non-thermal plasma modification

Abstract **#1101**

ANTAGONISTIC EFFECT OF MAGNESIUM HYDROXIDE NANOPARTICLE ON VASCULAR ENDOTHELIAL ACTIVATION INDUCED BY ACIDIC PLGA DEGRADATION PRODUCT

Dong Keun Han[P][1], Kyoung-Won Ko[1]

[1] *CHA university*

Corresponding author's email: dkhan@cha.ac.kr

Although drug-eluting stents (DESs) are mainly coated with biodegradable polymers such as poly(lactic-co-glycolic acid (PLGA) and poly(L-lactic acid) (PLLA), their acidic degradation products can affect homeostasis of adjacent tissue. Previously, we developed anti-inflammatory PLGA-based materials including magnesium hydroxide (MH) to relieve the side effects caused by PLGA degradation. However, the underlying molecular mechanism of its protective effects has not yet been clarified. Here, we demonstrated the molecular mechanism of vascular endothelial activation caused by PLGA byproducts. The degradation products of PLGA accumulated in HCAECs through the MCT1, followed by oxidative stress and the activation of the MAPK/NF-κB signaling pathway. Finally, the PLGA byproducts increased the expression of VCAM-1 as well as the secretion of pro-inflammatory cytokines. On the other hand, the addition of MH nanoparticles significantly diminished the activation of this pathological pathway and the expression of inflammation-related factors induced by PLGA byproducts. Furthermore, Mg2+ released from MH nanoparticles restored endothelial function in both intracellular and extracellular space. Taken together, MH nanoparticles prevent the accumulation of PLGA degradation products in HCAECs, thereby repressing the associated vascular endothelial activation. These findings on the biochemical mechanisms are expected to provide important clues for addressing the safety issues in nearly all biodegradable polymer-based implants.

Keywords

Drug-eluting stent (DES); Magnesium hydroxide (MH); Endothelial activation

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Abstract **#1102**

LOADING INDUCED SELF-HEATING OF ARTICULAR CARTILAGE AND ASSOCIATED SIGNALING MECHANISM

Naser Nasrollahzadeh$_P$[1,2], Lee A. Applegate[2], Dominique Pioletti[1]

[1] *Laboratory of Biomechanical Orthopedics-EPFL*

[2] *Lausanne University Hospital-CHUV*

Corresponding author's email: n.nasrlh@gmail.com

Chondrocytes in cartilage receive ample biophysical stimuli through normal physical activities. While temperature of knee intra-articular region is around 32°C at rest, a temperature evolution was recorded in vivo following joint loading during jogging[1]. This self-heating phenomenon is associated with the viscoelasticity of cartilage and conversion of the dissipated energy to heat following cyclic deformation[2]. Despite co-existence of thermo-mechanical cues in cartilage, it isn't known how the synergy of temperature increase and mechanical loading may contribute to chondrogenesis.

To study cartilage thermo-mechanobiology, a customized in vitro system was firstly developed to simulate respective biophysical cues in cartilage tissue. Combined or decomposed effects of temperature and loading on cells biophysical response were evaluated by using a poro-viscoelastic hydrogel[3] and a novel modular bioreactor. We also studied the contribution of calcium signaling and Transient Receptor Potential Vanilloid (TRPV) 4 channels to thermo-mechano transduction process.

The applied intermittent biophysical stimulus had a positive impact on chondrogenic response of human progenitor cells in all groups. In particular, expression of the transcription factor Sox9, one of the main regulators of early stage chondrogensis, was positively upregulated by application of combined thermo-mechanical cues. Moreover, a higher expression following thermo-mechanical stimulation was observed for main chondrogenic markers compared to thermal and mechanical cues alone. It was shown that calcium is a major signaling mechanism for translation of thermo-mechanical cues and there are parallel pathways to make this process more effective. TRPV4, as a thermo-mechano responsive ion channel, is a key mediator in transduction of loading induced self-heating.

Keywords

Thermo-mechanobiology; Cartilage Self-heating; Calcium signaling

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Abstract **#1103**

ANTI-OSTEOPOROTIC DRUGS FOR BONE BIOMATERIALS: PRECLINICAL INVESTIGATIONS IN HEALTHY AND OSTEOPOROTIC CONDITIONS

Hamdan Alghamdi[P][1,2], Amani Basudan[1], Marwa Shaheen[1], Abdurahman Niazy[3], Jeroen Beucken[4], John Jansen[4]

[1] *Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, Riyadh, Saudi Arabia*

[2] *Department of Dentistry - Biomaterials, Radboudumc, Nijmegen, The Netherlands.*

[3] *Department of Oral Medicine and Diagnostic Sciences, College of Dentistry, King Saud University, Riyadh, Saudi Arabia*

[4] *Department of Dentistry - Biomaterials, Radboudumc, Nijmegen, The Netherlands*

Corresponding author's email: dalghamdi@ksu.edu.sa

The general health in ageing population is a problematic issue in orthopedic and dental fields. Accordingly, the use of bone biomaterials has become a widely accepted treatment option. The healing of bone biomaterials is strongly influenced by local and systemic factors, which are related either to the materials itself or to the medical condition. For example, an altered bone metabolism due to osteoporosis can negatively affect bone-implant healing. In an attempt to enhance bone regeneration related to biomaterials in osteoporotic condition, several approaches have been reported in terms of material design, surgical techniques, and therapeutic additives. Nowadays, research efforts increasingly focus on the use of anti-osteoporotic drugs to promote bone response related to biomaterials in an impaired bone condition. Beside their role in the treatment of osteoporosis, systemic administration of anti-osteoporotic drugs is suggested to maintain a balance between bone resorption by osteoclasts and bone formation by osteoblasts around bone biomaterials.

In view of this, we have performed several preclinical studies to investigate the role of anti-osteoporotic drugs on bone healing around biomaterials in healthy and osteoporotic condition. The data of our preclinical studies indicate positive effects of anti-osteoporotic drugs on bone healing related to biomaterials (titanium implants and bone grafts). Although our results suggest that anti-osteoporotic drugs might reverse the negative impact of osteoporotic conditions on the peri-implant bone healing, more information is required from long-term implantation studies using standardized protocols for administration route, dosage and anti-osteoporotic drug before translational steps toward clinics.

Keywords

animal model; titanium implants; bone regeneration

Abstract **#1104**

DEVELOPMENT OF SPHEROID ENTRAPPED ALGINATE SCAFFOLD FOR SKIN TISSUE REGENERATION

SooJung Chae$_P$[1], JaeYoon Lee[1], WonJin Kim[1], JiUn Lee[1], MIji Yeo[1], YoungWon Koo[1], JuYeon Kim[1], Dongyun Kim[1], Hanjun Hwangbo[1], GeunHyung Kim[1,2]

[1] *Department of Biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea*

[2] *Biomedical Institute for Convergence at SKKU (BICS), Sungkyunkwan University, Suwon, 16419, South Korea*

Corresponding author's email: sjchae65@gmail.com

Tissue engineering aims to develop biological substitutes for the repair or complete replacement of injured tissues and organs [1]. In this field, spheroid has been drawing attention due to its regenerative properties. Spheroid defined as ball-shaped compacted cell aggregates consisting of monotypic or heterotypic living cells. Two interactions within the spheroid cause the improvement in biological properties compared to monolayer cultured cells. Cell-to-cell and cell-to-extracellular matrix interactions occurring within the spheroid mimics the in vivo environment. In this study, we successfully fabricated ASC spheroid entrapped scaffold using a combined system composed of 3D printing and electrospinning methods. The spheroids were positioned and entrapped in the pore of the scaffolds using electrospinning method. Angiogenic, wound healing-related gene expression analysis and tube formation assay were conducted to evaluate the properties of the fabricated scaffold. The ASC entrapped scaffold showed higher tube formation and better skin regeneration related gene expression compared to single cell entrapped scaffold. The angiogenic and wound healing gene expression results indicated that the hereby developed scaffold revealed to maintain the properties of spheroid. From those results, we conclude that the ASC spheroid entrapped alginate scaffold could be a new strategy for skin tissue regeneration.

Keywords

Spheroid; scaffold; skin regeneration

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Abstract **#1105**

ELECTRICAL STIMULATION VIA CONDUCTIVE POLYMER BED FOR HAIR FOLLICLE STEM CELL CULTURE

Lei Yan$_P$[1], Tatsuto Kageyama[2,1], Binbin Zhang[2,1], Junji Fukuda[1,2]

[1] *College of Engineering Science, Yokohama National University*

[2] *Kanagawa Institute of Industrial Science and Technology*

Corresponding author's email: yanlei6611@gmail.com

1. Introduction

Hair regenerative medicine has been investigated as a promising approach for the treatment of hair loss. Recent studies reported that transplantation of follicular stem cells resulted in regeneration of hair follicles and hairs. However, hair induction ability of dermal papilla cells (DPCs) is declining drastically after isolation from in vivo tissues and during expansion culture. In this research, we propose an electrical stimulation culture to recover the hair induction ability of DPCs using a conductive polymer polypyrrole (PPy).

2. Experimental

A culture device for electrical stimulation was built by electrochemically synthesizing PPy on a slide grass. DPCs at passage 4 were seeded onto the PPy membrane and applied electrical stimulation under several conditions. Gene expressions associated with hair follicle morphogenesis were evaluated. Furthermore, to examine de novo hair generation ability, the cells were collected after electrical stimulation and mixed with embryonic epidermal cells in a commercial non-cell-adhesive round-bottom 96 well plate (1). The aggregates were then transplanted on the back of mice. Hair shaft generation was evaluated for up to 3 weeks.

3. Results and discussion

The application of electrical stimulation significantly promoted alkaline phosphatase gene expression. The mixture of DPCs and epidermal cells exhibited typical morphological features of hair follicle germ in three days of culture. On intracutaneous transplantation into the backs of nude mice, the hair follicle germs prepared with the electrical stimulation efficiently generated hair follicles.

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Abstract **#1106**

HIGHLY POROUS INJECT-ABLE MERINGUE-LIKE 3D COLLAGEN STRUCTURE

YoungWon Koo[P][1], WonJin Kim[1], JiUn Lee[1], JuYeon Kim[1], Dongyun Kim[1], Hanjun Hwangbo[1], GeunHyung Kim[1,2]

[1] *Department of Biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea*

[2] *Biomedical Institute for Convergence at SKKU (BICS), Sungkyunkwan University, Suwon, 16419, South Korea*

Corresponding author's email: ethenkoo@gmail.com

Hydrogels have been used in tissue engineering extensively for cell container or niche. However, they have limitation in diffusible distances (0.2-0.4 mm) and researchers have tried to overcome this issue by mimicking the vascularized structure of our body [1-4]. However, vascularization of the artificial tissues usually needs additional processes and materials to compose a vasculature in the 3D structure. Here, we have developed a functional cell-laden bioink with meringue-like structure using collagen, which is the most common protein component in our body. Meringue has interconnected structure composed of air, liquid, and protein chains. With turbulent whipping process, air bubbles are introduced into the protein hydrogels and entrapped by the hydrophobic parts of the untangled protein chains, forming a highly macro/microporous structure. In the meringue-like structure, cells (MG63) could survive not only for more than 4 weeks, but also in about 2-mm depth or deeper, which is more than 10-fold deeper than the hydrogel diffusion limits (0.2 mm). In addition, this meringue-like bioink is injectable through gauge nozzles without structural destruction or cell damages. The advantages of the meringue bioink can be used to surpass the uppermost limit of bioprinting and tissue regeneration technologies.

Keywords

meringue; collagen; bioink

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Abstract **#1107**

DEVELOPMENT OF A 3D PIEZO-BIOMATERIAL TO STUDY CARTILAGE ELECTRO-MECHANOBIOLOGY

Lida Bagheri[P][1,2], Ahmad Ramazani S.A.[2], Naser Nasrollahzadeh[1], Dominique Pioletti[1]

[1] *Laboratory of Biomechanical Orthopedics-EPFL*

[2] *Sharif University of Technology*

Corresponding author's email: n.nasrlh@gmail.com

Despite increasing evidence that mechanical[1] and electrical[2] stimuli have positive effects on chondrogenesis, to date, the coupling effect of them has received a very limited attention. In articular cartilage, the electric potential is generated upon tissue deformation following the movement of the positive mobile ions away from the fixed negative charges[3]. Designing a 3D biomaterials with relatively stiff structure and capable for simulating the electrical signals of native cartilage is central to study cartilage electro-mechanobiology (stress-generated electric potential) in vitro. To address this challenge, we proposed a novel strategy to control both the mechanical and electrical performances of a hydrogel system. Accordingly, a 3D porous piezo-composite hydrogel with electro-mechanical properties within the range of cartilage is developed based on combination of fiber-reinforcement technique, electrospinning, and salt leaching methods. Despite traditional method used for in vitro electrical stimulation which are not biomimetic (e.g., using an external power supply), we employ electrospun piezoelectric polyvinylidene nanofibers in the hydrogel structure as an electroactive phase to generate electric signals upon mechanical loading. It is shown that incorporation of nanofibers into hydrogel networks significantly increase their mechanical properties and enhance cells adhesion and proliferation inside composite constructs thanks to the implemented nano-topographic structure. We present that piezoelectric effects promote cells growth and metabolic activity compared to respective control groups. The expression of chondrogenic markers following applied stimulation is also higher for the piezo-composite hydrogel compared to the pure hydrogels. This approach allow us to evaluate the synergistic effect of electro-mechanical cues on chondrocyte biological response.

Keywords

Composite hydrogels; Piezoelectric materials; Cartilage electro-mechanobiology

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Abstract **#1108**

FLEXIBLE FABRICATION OF BIOMIMETIC HYBRID MICROFIBERS CONSISTING CELL BEADS

Juyeon Kim$_P$[1], JaeYoon Lee[1], Miji Yeo[1], YoungWon Koo[1], WonJin Kim[1], JiUn Lee[1], SooJung Chae[1], Dongyun Kim[1], GeunHyung Kim[1,2]

[1] *Department of Biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea*

[2] *Biomedical Institute for Convergence at SKKU (BICS), Sungkyunkwan University, Suwon, 16419, South Korea*

Corresponding author's email: kelly2353@naver.com

The need for 3D culture of cells is emerging in the field of tissue regeneration. The three-dimensional (3D) cell culture systems are emerging as a powerful tool with enormous translational potential, especially for cancer and stem cell research. The 3D cell culture model can better reproduce not only morphology in the body but also cellular connectivity, polarity, gene expression and tissue structure, so it can have more physiological characteristics dynamically relevant and predictable compared to 2D models1. In this study, a microfluidic approach is presented that rapidly generates structure containing hydrogel micro bead that create 3D environment that enhances osteogenesis. Gelatin methacryloyl mixed with MG 63 cells and the prepared bio-ink were inserted with mineral oil into microfluidic. The microfluidics device was fabricated from polydimethylsiloxane to produce a water-in-oil emulsion containing GelMA and a photoinitiator in the aqueous phase and oil phase. Fabricated Gel-MA beads were crosslinked via UV light and inserted into second aqueous phase. The mixture was crosslinked by CaCl2 and alginate strut containing cell laden microbead was fabricated. The cell viability was measured by using live/dead staining. Rt-pcr test was performed to check cell differentiation. Enough cell viability (>90%) was observed after the bioprinting process. In this study, we developed a heterogeneous bioprinting strategy for the composite structure fabrication. The cell-laden heterogeneous strands were successfully printed by using the developed method. We expected that this new fabrication method can be used for the research about composite tissue and has the potential.

Keywords

Microfluidic; cell bead

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Abstract **#1109**

A PROTEOMIC APPROACH TO UNRAVEL THE ROLE OF THE EXTRACELLULAR MATRIX DURING KIDNEY ORGANOID MATURATION.

Virginie Joris[P][1]

[1] *Maastricht University*

Corresponding author's email: v.joris@maastrichtuniversity.nl

Two million people worldwide are dependent on dialysis with no option for renal transplantation. Alternatives are needed to address the medical demands and lessen the economic burden. One such alternative is a therapeutic engrafting of human induced pluripotent stem cell (iPSC)-derived kidney organoids to the dysfunctional kidney. We are generating kidney organoids, starting from human iPSCs, that consist of the major cell types present in adult human kidney. While robust differentiation protocols exist, relatively little is known about the events that lead to organoid maturation and vascularization. In contrast to what has been observed in vivo where transplanted kidney organoids further mature and vascularize, kidney organoids maintained in vitro fail this maturation step. It is known that the extracellular matrix (ECM) is a key component in embryonic kidney development and fetal maturation. Aberrant changes to its composition and organization have been linked to fibrosis and other diseases. We show that kidney organoids aged in vitro have an incomplete ECM that is associated with an abnormal spatial deposition. We performed a proteomic analysis on kidney organoids cultured for a prolonged culture time and found a specific change in the ECM composition with an increased expression of type 1a1, 2 and 6a1 collagen. Such an excessive accumulation of specific collagen types is a hallmark of renal fibrosis that causes a life-threatening pathological condition by compromising key functions of the human kidney. We believe that by controlling the expression of these proteins, we will be able to create a more mature kidney organoid.

Abstract **#1110**

REDUCING NON-RENAL CELL DIFFERENTIATION FORMING CHONDROGENIC CELL POPULATIONS IN KIDNEY ORGANOIDS.

Thomas Geuens[1], Virginie Joris[P][1]

[1] *Maastricht University*

Corresponding author's email: v.joris@maastrichtuniversity.nl

Kidney organoids derived from induced pluripotent stem cells (iPSC) are three-dimensional, mini kidney-like structures that aims to resemble human renal physiology. They form the important renal structures necessary for renal filtration such as glomeruli and proximal and distal tubuli. These organoids provide opportunities to study (genetic) renal diseases, renal development, and drug toxicity, but also as a potential clinical solution for patients with kidney failure. Two million people worldwide are dependent on dialysis with no option for renal transplantation. Alternatives are needed to address the medical demands and lessen the economic burden. One such alternative is a therapeutic engrafting of iPSC-derived kidney organoids to the dysfunctional kidney. However, these kidney organoids are not yet suitable for regenerative medicine as they bear non-renal cell differentiation, such as the appearance of chondrogenic cell populations upon aging and after in vivo transplantation. In order to enhance the renal phenotype of these kidney organoids we need to prevent the emergence of the non-renal phenotype. Therefore, we will mine existing single cell RNA sequencing datasets to form a hypothesis about which pathways are involved in forming the cells with the cartilage phenotype. These new data will allow us to screen for small molecule modulators of the identified pathway(s), which will first be tested for efficacy in vitro during iPSC differentiation and/or organoid development. In a final stage, our findings will be validated in vivo to check for the reduction of the cartilage phenotype and to determine if the renal phenotype has not been affected.

Abstract **#1111**

ESA 3D BIOPRINTER FACILITY FOR THE INTERNATIONAL SPACE STATION

Leonardo Surdo[P][1]

[1] *European Space Agency*

Corresponding author's email: leonardo.surdo@esa.int

3D printing is rapidly expanding: almost every week new printers and printing materials offering novel possibilities as well as new exciting applications appear. Within the life sciences and healthcare domains, numerous applications belong to the cell and tissue biology research as well as regenerative and personalized medicine applications.

3D bioprinting is steadily progressing on Earth and could find a further technological push and research achievements through its utilization in human space exploration.

Likewise, 3D cell cultures provide experimental models which more closely approach the structure and composition of organs and tissues. Studies with 3D cell culture models may be useful for understanding how processes in the whole organism are affected by microgravity or spaceflight conditions, which otherwise cannot be readily investigated in monolayer (2D) culture systems on Earth, animal or human subject studies.

ESA is planning to enhance the BIOLAB rack inside the ISS' Columbus module with a 3D bioprinting and 3D cell cultures capability to enable additional, state-of-art biology research in human spaceflight and to prepare biomedical applications for potential health emergency issues in anticipation of human long-term deep space exploration missions.

The ESA 3D bioprinting and 3D cell cultures capabilities will enable research investigations including, but not limited to fundamental and applied biotechnology, cell and tissue biology, regenerative and personalized medicine. The research performed with the 3D bioprinter and 3D cell cultures system on-board the ISS will build knowledge and technological advancement for future utilisation beyond the ISS, e.g. on Gateway, Moon and Mars human space exploration missions.

Abstract **#1112**

ADDITIVE MANUFACTURING OF AZ91D MAGNESIUM ALLOY BY 3D FIBER DEPOSITION

JiaPing Li[P][1], Moroni Lorenzo[2], Habibovic Pamela[3]

[1] MERLN institure. Maastricht University. The Netherlands

[2] MERLN institure. Maastricht university,The Netherlands

[3] MERLN Institure. Maastricht Univeruty,The Netherlands

Corresponding author's email: jiaping.li@maastrichtuniversity.nl

Magnesium(Mg) alloys have attracted attention in biomedical applications due to their biodegradability and satisfying mechanical properties (1). Mg alloy implants have been shown to reduce stress shielding when compared to other non-degradable metal implants due to its low young's modulus. Mechanical properties of the widely used degradable polymers such as polylactic acid, polycaprolactone are about a half of the those of Mg alloys (2). Recently, a few groups attempted additive manufacturing (AM) of Mg alloys and showed promising results (3). Nevertheless, AM of Mg remains challenging due to its high vapor pressure and high oxidation affinity (4). In this study, 3D fiber deposition (3DF) was applied as AM technology to fabricate porous AZ91D scaffolds by using a slurry of MgAl powder at RT in a cost effective way. First, binder system for MgAl slurry was developed to avoid any reaction with Mg. Second, the rheological properties of slurry were optimized to allow printing and avoid sagging and shape deformation of the fibers. Finally, debinding and sintering process was set up to prevent contamination during sintering. The resulting 3D porous MgAl scaffolds had hierarchical structure with interconnected pores and fusion between metal particles. The chemical analysis indicated the no contamination occurred during debinding and sintering process. The compressive strength and Young's modulus with porosity of 30% were 89±9 MPa and 0.6±0.12GPa, respectively. The in vitro degradation study of the scaffolds is ongoing. 3DF may be a useful methods for manufacturing 3D porous MgAl scaffolds for biomedical application.

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Abstract **#1113**

FABRICATION OF MICRO-CHANNELED NATIVE-DERIVED HYDROGEL/BIOCERAMICS COMPOSITE SCAFFOLD FOR DAMAGED BONE REMODELING

Hanjun Hwangbo[P][1], Miji Yeo[1], JaeYoon Lee[1], YoungWon Koo[1], WonJin Kim[1], JiUn Lee[1], SooJung Chae[1], Jiyoung Hong[1], JuYeon Kim[1], Dongyun Kim[1], GeunHyung Kim[2,3], Hyeongjin Lee[2]

[1] *Department of biomechatronic Engineering, Sungkyunkwan University (SKKU),*

[2] *Department of biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea*

[3] *Biomedical Institute for Convergence at SKKU (BICS), Sungkyunkwan University, Suwon, 16419, South Korea*

Corresponding author's email: hhwangbokorea@gmail.com

Scaffolds consisting of native tissue-derived hydrogel is gradually approaching the golden standard for tissue engineering for its close approximation to the target tissue via mirroring the architecture and intricate composition. However, scaffold with appropriate geometries and materials are often challenging and difficult. To overcome this issue, we introduced a micro-channeled native-derived hydrogel/biceramics composite scaffold. Briefly, a sacrificial fibrous PCL structure was fabricated via conventional 3D printing. Then, followed by coating the sacrificial structure with native-derived hydrogel and bioceramics, the PCL fibers were removed for achieving the micro-channeled composite scaffold (mCCS). The mCCS showed highly porous mesh structure consisting of micro-channeled structs. The channels evoked high capillary pressure, resulting in efficient angiogenic properties within the scaffold. Consequently, immunofluorescent staining results revealed that angiogenesis was achieved in the micro-channels, while osteogenesis was achieved on the surface of the scaffold. Based on the promising in vitro results, the purposed mCCS would increase the efficacy of bone tissue regeneration.

Keywords

bioceramic; native derived

Abstract **#1115**

CARDIAC PROGENITOR CELLS DELIVERY WITHIN CROSSLINKED POLYPYRROLE INCORPORATED CARDIOGEL RESTORED FUNCTION IN POST ISCHEMIC RAT HEART

Sara Pahlavan[1], Hassan Ansari[1], Elena Mahmoudi[2], Maryam Barekat[1], Sarah Rajabi[1], Sara Pahlavan[3], Melika Kashani[P]

[1] *Royan Institute for Stem Cell Biology and Technology*

[2] *Harvard Medical School*

[3] *Royan Institute for Stem Cell Biology and Technology *Corresponding author*

Corresponding author's email: melika.kashani73@gmail.com

Myocardial infarction (MI) causes irreversible damage to the heart, resulting in impaired heart function. Recently, cardiac tissue engineering has emerged as a novel therapeutic approach for functional repair post-MI. Specifically, electroconductive bioengineered tissues could improve the effects of cardiac cell therapy. In this study, we aimed to use a mechanically stable, crosslinked form of polypyrrole (PPy) incorporated cardiogel (CG) for in vivo transplantation of cardiac progenitor cells (CPCs) in rat hearts post-MI. The crosslinked CG-PPy scaffold (P1) showed a three-dimensional (3D) interconnected porous structure in which vital extracellular matrix (ECM) components were preserved while attained appropriate mechanical properties as well as significantly greater electrical conductivity (9-fold) compared to a crosslinked CG scaffold (C1). Transplantation of CPC-loaded P1 on the rat hearts post-MI led to substantial improvement of some cardiac functional and morphological features such as left ventricular ejection fraction (LVEF), fractional shortening (FS) and left ventricular inner diameter at systole (LVIDs) compared to the vehicle receiving group (Ctrl), while forming considerably lower fibrotic tissue. Grafted cells revealed cardiomyocyte–characteristics as per staining with antibodies against human cardiac troponin T (hcTNT) and human connexin43 (hCx43). Furthermore, higher number of α-SMA positive vessels were observed in the heart sections of P1-CPC transplanted rats compared to all other treatments four-week post-surgery. These findings highlight the potency of a crosslinked CG-PPy scaffold as CPCs carrier for regenerative therapy of the heart post-MI.

Abstract **#1116**

MICROSPHERES: A POWERFUL STRATEGY TO TUNE THE BIOLOGICAL PERFORMANCE OF BIOINKS

Mar Bonany$_P$[1,2], Montserrat Espanol[1,2], Maria-Pau Ginebra[1,2,3]

[1] *Biomaterials, Biomechanics and Tissue Engineering Group, Dept. of Materials Science and Engineering, Universitat Politècnica de Catalunya (UPC), Barcelona, Spain*

[2] *Barcelona Research Centre for Multiscale Science and Engineering, UPC, Barcelona, Spain*

[3] *Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain*

Corresponding author's email: mar.bonany@upc.edu

Alginate is an attractive candidate in the formulation of bioinks. However, the lack of cell adhesive moieties requires additional processing strategies that usually affect the rheological properties of the bioink and, in turn, their manipulation and in vitro performance. Therefore, the main goal of the present work was to design simple, robust and tuneable cell-laden inks through the incorporation of microspheres of different nature for bone regeneration applications.

Three types of microspheres (i.e. gelatine, gelatine containing hydroxyapatite nanoparticles and hydroxyapatite) were synthesised and incorporated into an alginate/gelatine ink loaded with cells. Immediately after, the mixtures were extruded, cross-linked and incubated in cell culture media for different days. Cell viability and proliferation were assessed at different time points by live/dead and Alamar Blue assays, respectively. Furthermore, the differentiation to osteoblastic lineage was studied by measuring gene expression of osteogenic markers through RT-qPCR and immunofluorescence assays.

The results confirmed the cytocompatibility of all bioinks. On the one hand, the incorporation of gelatine microspheres promoted cell migration and adhesion at short time points, as well as cell proliferation throughout the whole experiment. On the other hand, hydroxyapatite-containing microspheres induced the differentiation of the cells to osteoblastic phenotype for both, early and late genes.

In conclusion, we have developed robust and tuneable alginate-based bioinks with improved biological features by simply adding microspheres of different composition in the ink formulation. This strategy efficiently overcomes the limitations arising from chemical modifications of current alginate bioinks and can easily be applied to other biomedical fields.

Abstract **#1118**

RECREATING THE BREAST CANCER MICROENVIROMENT USING ELASTIN-LIKE RECOMBINAMER HYDROGELS

Barbara Blanco-Fernandez[P][1], Arturo Ibañez-Fonseca[2], Doriana Orbanic[2], Soledad Perez-Amodio[1,3], José Carlos Rodriguez-Cabello[2], Elisabeth Engel[3,4,1]

[1] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10–12, 08028 Barcelona, Spain.

[2] BIOFORGE Lab, CIBER-BBN, University of Valladolid, Paseo de Belén 19, 47011 Valladolid, Spain.

[3] Materials Science and Metallurgical Engineering, Polytechnical University of Catalonia (UPC), Eduard Maristany 16, 08019 Barcelona, Spain.

[4] CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain.

Corresponding author's email: bblanco@ibecbarcelona.eu

Introduction. The low success of anti-cancer drugs in clinical trials evidence that current 2D in vitro models used in preclinical studies cannot fully mimic the 3D tumor microenvironment. Therefore, more predictive 3D in vitro models are needed. Among them, cancer spheroids (cS) recapitulate many tumor characteristics. Peptide hydrogels (HGs) have been shily explored as platforms for cS production. Particularly, elastin-inspired peptides are an interesting approach for cancer research due to their tunable properties. Elastin-like recombinamers (ELR) self-assemble above certain temperatures forming biocompatible gels, that can be cross-linked by click chemistry.[1]

The aim of this work is to assess the potential of ELR HGs to create breast cancer cS for drug screening.

Methods. Breast cancer (MCF-7, MDA-MB-231) or non-tumor breast (MCF10A) cells were encapsulated within ELR hydrogels. Cells were dispersed in an MMP-sensitive ELR with cyclooctine groups, and then mixed with an ELR carrying azide groups and RGD. Cell proliferation, viability, cell morphology and ECM deposition were evaluated. Doxorubicin IC50´s were measured after 48h in hydrogels and 2D cultures.

Results and discussion. Hydrogels were biocompatible and enabled a homogeneous cell distribution. MCF-7 and MCF10A formed cS whereas MDA-MB-231 formed cell networks. All cell types deposited ECM proteins after 7d. Cells cultured in the hydrogels were more resistant to doxorubicin than the 2D counterpart.

Conclusions. ELR HGs are a promising material for developing in vitro breast cancer models for drug screening.

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Keywords

hydrogels; spheroids; breast cancer

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Abstract **#1119**

EXPLORING THE ROLE OF PERIPHERAL MYELIN PROTEIN 22 EXPRESSION IN ORDERED MEMBRANE DOMAINS OF SCHWANN CELL DIFFERENTIATED DENTAL PULP STEM CELLS AND HOW IT AFFECTS CELL-MATRIX INTERACTIONS

Tim Vangansewinkel[1], Karen Libberecht[1], Pascal Gervois[1], Ronald Driesen[1], Robert Prior[2,3], James Phillips[4,5], Ludo Van Den Bosch[2,3], Esther Wolfs[1], Ivo Lambrichts[1]

[1] *Cardio and organ systems, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium*

[2] *Department of Neurosciences, KU Leuven - University of Leuven, Experimental Neurology and Leuven Brain Institute (LBI), Leuven, Belgium*

[3] *Center for Brain & Disease Research, Laboratory of Neurobiology, VIB, Leuven, Belgium*

[4] *UCL Centre for Nerve Engineering, University College London, London, United Kingdom*

[5] *Department of Pharmacology, UCL School of Pharmacy, London, United Kingdom*

Corresponding author's email: tim.vangansewinkel@uhasselt.be

New therapeutic strategies to establish repair of injured peripheral nerves are desperately needed. For this purpose, our group developed Engineered Neural Tissue with Schwann cell differentiated human dental pulp stem cells (SC-hDPSC) embedded in an aligned collagen hydrogel 1, 2. The use of SC-DPSCs as a cell source for nerve repair has several advantages but there is a need to better understand how they interact with the extracellular matrix (ECM) and how this affects their myelinating behavior. We aim to investigate the role of peripheral myelin protein 22 (PMP22) in the interaction of SC-hDPSCs with an endoneurial-like ECM.

SC-hDPSC were cultured on 2D coated surfaces and by performing immunostainings combined with confocal- and super resolution STORM microscopy we showed that PMP22 is expressed in lipid rafts of the plasma membrane. Live cell/TIRF imaging experiments with cholera toxin b showed that these lipid rafts are highly dynamic. We also observed a strong colocalization between PMP22 and integrin β1, α6, and β4 in the membrane of SC-hDPSC. In addition, SC DPSC cultured in aligned or free floating hydrogels strongly interact with collagen I fibres via these integrins, which was confirmed macroscopically and with label-free second harmonic generation microscopy.

In conclusion, we showed that PMP22 is expressed in ordered regions in the SC-hDPSC membrane together with integrins that are important for cell interactions with the ECM and in myelination. This implicates that PMP22 may play a role in controlling myelination, but this will be explored in future experiments.

Keywords

Dental stem cells; Schwann cells; Extracellular matrix

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Abstract **#1120**

DEVELOPING A SOFT-HARD COMPOSITE SCAFFOLD USING ELECTROSPINNING AND 3D PRINTING

Rand Alkaissy$_P$[1], Pierre-Alexis Mouthuy[1], Andrew Carr[1]

[1] *Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS)*

Corresponding author's email: rand.alkaissy@ndorms.ox.ac.uk

Rotator cuff tendon tears are one of the most common injuries of the musculoskeletal system, resulting in 40,000 surgical re-attachment repairs in the United States alone[1]. Re-tearing following rotator cuff repair is a significant clinical problem, with a failure rate of up to 40%[1], notably at the transition from bone to tendon (enthesis)[2]. The aim of this project is to develop a biphasic scaffold consisting of a soft and hard component, which can recapitulate this interface.

To achieve this, a novel manufacturing approach was developed, combining 3D printing and electrospinning. A porotype scaffold was created by bonding together polydioxanone (PDO) fibres and polycaprolactone (PCL) lattice structures. Firstly, PDO fibres were continuously produced by electrospinning on a metal wire collector[3,4], after which PCL lattice structures were extruded at a temperature of 120 °C on top of the pre-assembled electrospun fibres to complete the scaffold. No additional post-processing was required to use the scaffolds for further characterization.

SEM imaging of the PDO fibers showed an alignment that resembles fibrils within a tendon. Micro-CT imaging of the prototype scaffold revealed intact PDO fibers within the 3D printed construct. Preliminary mechanical testing showed an ultimate failure force at 47.4 +/- 2.2 N. Biocompatibility of the scaffold will be tested in a second phase.

While preliminary results show mechanical properties not yet representative of the full rotator cuff force, we suggest that combining electrospinning and 3D printing is a feasible and easy approach with room for optimization to create a mechanically robust soft-to-hard biphasic scaffold.

Keywords

rotator cuff; electrospinning; 3D printing

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Abstract **#1121**

TISSUE ENGINEERED 3D FIBER-DEPOSITED SCAFFOLDS FOR OSSICULAR CHAIN REPLACEMENT PROSTHESIS

Mario Milazzo$_P$[1], Carlos Mota[2], Stefano Berrettini[3], Anbuselvan Dharmarajan[4,5], Sunil Puria[4,5], Lorenzo Moroni[2], Serena Danti[1,3,6]

[1] Laboratory for Atomistic and Molecular Mechanics (LAMM), Dept. of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge (MA), United States of America

[2] Institute for Technology Inspired Regenerative Medicine (MERLN), Complex Tissue Regeneration Department, Maastricht University, Maastricht, The Netherlands

[3] Laboratory of Temporal Bone Dissection and Otologic Tissue Engineering (OtoLab), Department of Surgical, Medical, Molecular Pathology and Emergency Medicine, University of Pisa, Pisa, Italy

[4] Eaton-Peabody Laboratory, Massachusetts Eye and Ear, Boston (MA), United States of America

[5] Department of Otolaryngology & Head and Neck Surgery, Harvard Medical School, Boston (MA), United States of America

[6] Dept. of Civil and Industrial Engineering, University of Pisa, Largo L. Lazzarino 2, Pisa, Italy

Corresponding author's email: milazzo@mit.edu

Conductive hearing loss affects middle ear functionality in more than 5% of the population worldwide. To replace damaged ossicles, surgical approaches use piston-like prostheses, made of biological grafts or alloplastic biomaterials, to restore the mechanical continuity between the tympanic membrane and the oval window (1). However, many of these fall off after a period of time requiring revision surgery. We present a new generation of partial ossicular replacement prostheses (PORPs) shaped like scaffolds made of poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT) copolymer, and fabricated via three-dimensional fiber deposition. Dimensional and porosity features of the scaffolds are characterized. Human mesenchymal stromal cells are then osteo-differentiated on PORP-scaffolds using serum-free and xeno-free media to investigate the capability of such structures to be properly hosted by native tissues under a translational approach. Cell viability result stable up to 3 weeks, show successful osteo-differentiation of the stem cells in the scaffolds. This indicates the potential for minimizing the risk of prosthesis extrusion and minimizing revision surgery. We use Laser Doppler Vibrometry to assess performance of the prostheses in response to sound stimulation (2). PORPs are placed by an otologist on 4 human cadaveric temporal bones, in a frequency range between 100 Hz and 20 kHz. Stapes motion shows a good match against intact middle-ear outcomes with little inter-ear variability, especially at frequencies below 3 kHz. These results pave the way to a promising tissue engineering approach for ossicular replacement that can be used as a reliable long-term surgical alternative to the current standard of care.

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Abstract **#1122**

DEVELOPMENT OF A MODEL FOR THE INVESTIGATION OF SURFACE INTERACTIONS BETWEEN ORAL MEDICINES AND THE OESOPHAGUS

Anna Johnston[P][1], Hannah Batchelor[2], Marie-Christine Jones[3], Anita Ghag[1]

[1] *School of Chemical Engineering, University of Birmingham, UK*

[2] *Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, UK*

[3] *School of Pharmacy, University of Birmingham, UK*

Corresponding author's email: AXJ976@student.bham.ac.uk

In vitro models of the human gastrointestinal (GI) tract are often used for study in the food and pharmaceutical industries. A range of models for most organs of the GI tract have been developed. Current in vitro oesophagus models are often simplistic and do not simulate physiological conditions accurately 1. This project aims to develop a model of the human oesophagus that can be used to study the passage of orally administered medicines. In order to do this a material that mimics the surface of the oesophagus tissue needs to be developed. The innermost surface of the oesophagus has a thin basement membrane network of fibres 2. Electrospinning has been used to replicate this layer. Data was obtained on the mechanical and surface properties of porcine oesophagus, which is physiologically similar to that of humans. An electrospun polyurethane (PU) material was produced and combined with a gel phase (gelatin). The average fibre diameter of the electrospun PU was 128.1nm which, while higher that the reported average fibre diameter of the oesophageal basement membrane fibres (66nm), was within the reported acceptable range of 28-165nm 2. Mechanical testing of the electrospun PU showed that it had significantly different properties than the porcine oesophagus. However, when the electrospun PU is combined with a gelatin phase preliminary observations show a large change in the mechanical properties of the bulk material and results in a material which appears to share more textural and surface characteristics with porcine oesophagus tissue.

Keywords

Polyurethane electrospinning; Oesophagus; In vitro modelling

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Abstract **#1124**

CONTROLLED ACTIVATION OF PREFABRICATED A-TRICALCIUM PHOSPHATE CEMENT PASTES STABILIZED BY SODIUM PYROPHOSPHATE

Katrin Hurle-P[1], Jan Weichhold[2], Friedlinde Götz-Neunhoeffer[1], Uwe Gbureck[2]

[1] *GeoZentrum Nordbayern, Mineralogy, Friedrich-Alexander-University of Erlangen-Nürnberg (FAU)*

[2] *Department for Functional Materials in Medicine and Dentistry, University Hospital Würzburg*

Corresponding author's email: katrin.hurle@fau.de

Calcium phosphate cements are frequently applied as bone substitutes due to their excellent biocompatibility. For successful clinical application, easy handling at the surgery is important. The mixing procedure can be effectively facilitated by the application of prefabricated cement pastes: Instead of mixing a liquid and a powder, a solution and a suspension are mixed, which can be assembled in one special syringe [1].

One prerequisite of this approach is the successful stabilization of a cement powder suspension in parts of the mixing liquid. In the present study, a suspension of reactive α-tricalcium phosphate powder in water was successfully stabilized up to 4 weeks at room temperature by sodium pyrophosphate (PP) in a concentration of 0.05 wt.%. Controlled activation of the hydration to calcium-deficient hydroxyapatite was accomplished by addition of a Na2HPO4/NaH2PO4 (Na2/Na) solution (weight ratio 4:1; overall concentration 30 wt.%). For systematic assessment of the activation mechanism, including the effect of the PP concentration and the amount of Na2/Na added, the hydration was studied by isothermal calorimetry, quantitative in-situ X-ray diffraction and Gillmore needle measurements at 37 °C.

Increasing the PP concentration lead to systematic retarding of the setting reaction at constant Na2/Na amount. An elevated quantity of Na2/Na addition at constant PP concentration resulted in acceleration of the hydration. These results indicate that the setting mechanism is adjustable for clinical needs by proper selection of the PP concentration and amount of Na2/Na added.

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Abstract **#1125**

A TISSUE ENGINEERED PRODUCT CONSISTING IN RPE DERIVED FROM HUMAN EMBRYONIC STEM CELLS DISPOSED ON HUMAN AMNIOTIC MEMBRANE FOR RETINITIS PIGMENTOSA CLINICAL TRIAL

Christelle Monville[P1], Karim Ben M'Barek[2], Stéphane Bertin[3], Elena Brazhnikova[4], Céline Jaillard[4], Walter Habeler[2], Alexandra Plancheron[2], Claire-Maëlle Fovèt[5], Joanna Demilly[5], Mohamed Jarraya[6], Ana Bejanariu[2], Thierry Bordet[7], José-Alain Sahel[8], Marc Peschanski[2], Olivier Goureau[4]

[1] *INSERM/UEVE UMR861, Istem*

[2] *CECS/Istem*

[3] *CHNO des Quinze-Vingt, DHU Sight Restore, INSERM-DGOS CIC1423*

[4] *Institut de la Vision, INSERM U968, Sorbonne Universités, CNRS, UMR 7210*

[5] *CEA-MIRCen*

[6] *Banque de Tissus Humains, Hôpital Saint-Louis, AP-HP*

[7] *Eyeventis*

[8] *CHNO des Quinze-Vingt, DHU Sight Restore, INSERM-DGOS CIC1423; Department of Ophtalmology, University of PittsburgInstitut de la Vision, INSERM U968, Sorbonne Universités, CNRS, UMR 7210*

Corresponding author's email: cmonville@istem.fr

In developed countries, retinal degenerative diseases affecting Retinal Pigmented Epithelium (RPE), including Age-related Macular Dystrophy and inherited retinal diseases such as Retinitis Pigmentosa (RP), are the predominant causes of human blindness worldwide. So far, there is no cure for such diseases. We have developed a cell therapy medicinal product based on our expertise in tissue engineering and in the manipulation of pluripotent stem cells. This novel tissue engineered product (TEP) consists in RPE cells derived from clinical grade human embryonic stem cells disposed on a biocompatible substrate allowing the formation of a 3D functional sheet, suitable for transplantation. After functional validation in a rodent model of RP, we have tested the safety of the surgery and local tolerance in non-human primates (NHP).

A specific device was developed and non-human primates (NHP, n=6) were transplanted in one eye (right eye) with the TEP in the macular region. Retinal integrity and functionality were assessed at different time points (week 1, 2, 4, 6 and 7). Inflammation was also assessed. At the end of the experimental period, histological analysis was performed to evaluate the correct location and integration of the TEP within the host retina.

We have shown in NHP that our surgical method of implantation was safe and did not provoke any local inflammation or retinal deterioration. Morphologic and histologic studies indicated that RPE cells were integrated into the host retina and were able to interact with photoreceptors. These results allowed us the start of clinical trial in sept 2019 (NCT NCT03963154).

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Abstract **#1126**

ECM-MICROFIBER LADEN BIOINKS ENABLE SPATIAL CONTROL OF CAPILLARY FORMATION IN 3D BIOPRINTED CONSTRUCTS

Margo Terpstra$_P$[1], Jinyu Li[2], Anneloes Mensinga[1], Mylène de Ruijter[1], Michiya Matsusaki[2], Jos Malda[1,3], Riccardo Levato[1,3]
[1] Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, 3584CX, Utrecht, The Netherlands.
[2] Division of Applied Chemistry, Graduate School of Engineering, Osaka University, Suita, Osaka, 565-0871, Japan
[3] Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, 3584CX, Utrecht, The Netherlands.
Corresponding author's email: m.l.terpstra-4@umcutrecht.nl

Most tissues in our body are fully vascularized which facilitates the gas and nutrient exchange from the blood circulation. Menisci, however, consist of a vascularized and non-vascularized region. In tissue engineering, mimicking the spatial distribution of vascular components is paramount. In fact, capillary ingrowth into non-vascularized tissues can lead to tissue matrix alterations and subsequent pathology. Three-dimensional (3D) bioprinting has the potential to reproduce tissues with anisotropic features. Nevertheless, recreating a construct with stable vascularized and non-vascularized regions has not been achieved to date.

Previously, it was shown that fibrin bioinks with type I collagen microfibers facilitate capillary and lumen formation of HUVECs (1). Here, we additionally developed cartilage matrix microfibers, normally involved in preserving the avascular nature of cartilage (2,3). We demonstrate that they successfully inhibited HUVEC capillary formation. Through supplementation of two fibrin-based bioinks with type I collagen or cartilage matrix microfibers, we 3D bioprinted complex structures with distinct regions that facilitate or inhibit vascularization. Constructs were 3D printed in a gellan gum microparticle-based suspension bath and showed a filament thickness of 350 micron. Four days after printing, the HUVECs showed network formation.

This study demonstrates the spatial-specific formation of capillaries in a 3D bioprinted construct. With this, we contribute to the development of biomimetic meniscal grafts. Moreover, this setup can serve as a platform for generating tissue constructs with vascularized and non-vascularized regions. These bioinks could be utilized for in vitro models for studying effects of new drugs, or mechanisms of vascularization.

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Abstract **#1127**

INFLUENCE OF CU2+-DOPING IN B-TCP ON THE HYDRATION KINETICS OF BRUSHITE CEMENT

Karla Späth[P][1], Katrin Hurle[1], Friedlinde Goetz-Neunhoeffer[1]

[1] *Geozentrum Nordbayern, Mineralogy, Friedrich-Alexander University Erlangen-Nürnberg*

Corresponding author's email: karla.spaeth@fau.de

One example for brushite (CaHPO4·2 H2O) formation is the reaction of monocalcium phos-phate monohydrate (MCPM, Ca(H2PO4)2·H2O) and β-tricalcium phosphate (β-TCP, β-Ca3(PO4)2), which react with H2O according to (1). The use of Cu2+ as dopant for β-Ca3(PO4)2 leads to increased antibacterial activity, hence preventing inflammation [1] and has positive effects on angiogenesis and wound healing [2].

Ca(H2PO4)2·H2O + β-Ca3-xCux(PO4)2 + 7 H2O → 4 CaHPO4·2 H2O (1)

The effect of Cu2+ doping in β-TCP on the hydration was investigated by isothermal heatflow calorimetry, powder and in-situ XRD, ICP-MS measurements of the pore solution and 1H-NMR for determination of different proton mobility in free H2O and formed phases. β-TCP was mixed with MCPM with a molar ratio of β-TCP/MCPM = 1.63 and a water to solid ratio of 0.3 ml/g. Phytic acid (IP6, C6H6(OPO3H2)6) was used as a setting retarder. The measure-ments were conducted at 23 °C and selected mixtures were measured at 37 °C and 5 °C. β-TCP with Cu2+ contents between 0 and 5 mole-% were used. Heatflow calorimetry and in-situ XRD measurements showed an increasing retardation of the reaction with increasing amount of Cu2+ in β-TCP. Brushite and small amounts of monetite were detected as reaction products. The samples with Cu2+ show also a secondary phase with a strong preferred orientation, which is assumed to be a Cu2+ containing hydrate phase.

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Abstract **#1128**

DESIGN AND DEVELOPMENT OF A NEW MICROFLUIDIC VASCULARIZED PLATFORM TO MODEL THE HUMAN BLOOD BRAIN BARRIER

Vita Guarino[P][1,2], Elisabetta Perrone[2], Alessandra Zizzari[2], Monica Bianco[2], Antonella Stanzione[1,2], Francesca Gervaso[2], Giuseppe Gigli[1,2], Lorenzo Moroni[3,2], Valentina Arima[2]

[1] *Università del Salento, Department of Mathematics and Physics "E. De Giorgi" via Arnesano, 73100 Lecce, Italy*

[2] *CNR NANOTEC – Institute of Nanotechnology, c/o Campus Ecotekne, Via Monteroni, 73100 Lecce, Italy*

[3] *Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, department of complex tissue regeneration, 6229ER, Maastricht, The Netherlands*

Corresponding author's email: vita.guarino@unisalento.it

Organ-on-Chips (OoCs) are nowadays considered the nascent technology to replace animal models, in compliance with the principles of the 3Rs (Replacement, Reduction and Refinement) developed over 50 years ago [1]. Although that goal is still far off, their use might illuminate differences between animal models and humans, with important outcomes in drug screening, disease model and regenerative medicine [2]. In all these fields, vascularization is a key aspect and OoCs are particularly attractive to realize capillary vessels. Particularly in the brain, vascularization has exceptional features, which are represented in the blood-brain barrier (BBB). Using OoCs to develop a model of the human BBB is considered an interesting way to overcome the limitations of rodent models and transwell systems, by combining the handiness of an in vitro model with the complexity of a living organ, while reducing reagents consumption [3]. Our work is focused to develop a human BBB-on-chip model based on a continuously perfused microfluidic platform, recreating the exposure of the cells to different shear stresses, which can upregulate genes associated with junctional proteins and transporters, enhancing barrier integrity [4]. The chip design is based on a double-layer layout that puts in contact a fluidic compartment with endothelial cells ("blood side") to a static compartment with brain cells in a 3D matrix ("brain side") reproducing the three-dimensional environment of the brain parenchyma. In addition, our model allows the deposition of 3D brain cells by bioprinting technique for better controlling their spatial distribution.

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Abstract **#1129**

CONTROLLED RELEASE OF OSTEOBLAST-DERIVED EXTRACELLULAR VESICLES FROM AN INJECTABLE CHITOSAN-COLLAGEN COMPOSITE HYDROGEL TO PROMOTE BONE FORMATION

Kenny Man$_P$[1], Mathieu Brunet[1], Maria Fernandez-Rhodes[2], Owen Davies[2], Sophie Cox[1]

[1] *University of Birmingham*

[2] *Loughborough University*

Corresponding author's email: k.l.man@bham.ac.uk

For bone tissue engineering, the use of extracellular vesicles (EVs) are emerging as a promising acellular approach compared to cell-based therapies [1]. Despite their promise, the short half-life of these naturally-derived nanoparticles following systemic administration hinders their therapeutic potential. Therefore, this study aimed to develop an injectable chitosan-collagen composite hydrogel capable of controlling the release of osteoblast-derived EVs to promote bone regeneration.

Chitosan-collagen composites were fabricated at ratios of 100/0, 65/35, 25/75 and 0/100 wt%. The hydrogels gelation time, compressive modulus and pore size were characterised. Osteoblast-derived EVs were incorporated within the different composites and their release kinetics were determined using the CD63 ELISA. The size, morphology and concentration of released EVs were assessed via dynamic light scattering, nanoparticle tracking analysis and transmission electron microscopy. The osteogenic differentiation of human bone marrow stromal cells (hBMSCs) encapsulated within the EV-functionalised hydrogel was evaluated by qPCR, biochemistry and histological analysis.

The presence of collagen within the composite hydrogel significantly enhanced compressive modulus and reduced gelation times and pore size. EVs release kinetics was collagen dependent, where greater the proportion of chitosan in the composite, resulted in increased EV release. In monolayer, the functional activity of hydrogel released EVs was confirmed with enhanced hBMSCs proliferation and migration. Importantly, EV-functionalised composite hydrogels significantly promoted encapsulated hBMSCs osteogenic differentiation when compared to the EV-free gel during osteogenic culture.

These findings demonstrated the development of an injectable chitosan-collagen composite capable of controlling the release of osteoblast-derived EVs as a novel acellular tool to promote bone regeneration.

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Abstract **#1130**

INJECTABLE CALCIUM SULFATE-CALCIUM POLYPHOSPHATE PUTTY FOR BONE BLEEING CONTROL AND BONE REGENERATION

Huan Zhou[P][1], Lei Yang[1]

[1] *Hebei University of Technology*

Corresponding author's email: zhouhuan@hebut.edu.cn

Bone bleeding is a common symptom in orthopedics. Development of biomedical materials for the intraoperative hemorrhage control and postoperative bone regeneration are crucial for the safety and recovery of patients. Injectable putty composed of a core-shell structured calcium sulfate-calcium polyphosphate granules and cellulose gel was developed to fill the bleeding site of bone and locally enhance bone regeneration. The former granule was prepared using calcium sulfate cement as templates, and the latter gel was prepared via mixing cellulose and water. Taking the advantages of calcium polyphosphate in being hydrolyzed by osteoblast released alkaline phosphatase to generate energy, phosphate ions, along with the fast resorption rate of calcium sulfate to provide calcium ions and space, the implanted granules after hemostasis can stimulate bone regeneration. This novel injectable putty is expected to be an alternative to bone wax for bleeding bone treatment.

Abstract **#1131**

CRANIOFACIAL PERIOSTEUM-DERIVED CELLS ARE A POTENTIAL SOURCE FOR BONE TISSUE ENGINEERING

Lisanne Groeneveldt[p][1,2,3], Tim Herpelinck[1,2], Marina Maréchal[1,2], Constantinus Politis[4,5], Wilfred van IJcken[3,6], Danny Huylebroeck[3,7], Lies Geris[1,2,8,9], Eskeatnaf Mulugeta[3], Frank Luyten[1,2,7]

[1] *Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium*

[2] *Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium*

[3] *Department of Cell Biology, Erasmus University Medical Center, Rotterdam, The Netherlands*

[4] *OMFS IMPATH research group, Department of Imaging and Pathology, KU Leuven, Belgium*

[5] *Oral and Maxillofacial Surgery, University Hospitals Leuven, Leuven, Belgium*

[6] *Biomics-Genomics, Erasmus University Medical Center, Rotterdam, The Netherlands*

[7] *Department of Development and Regeneration, KU Leuven, Belgium*

[8] *Biomechanics Research Unit, GIGA-R In Silico Medicine, Université de Liege, Liège, Belgium*

[9] *Biomechanics Section, KU Leuven, Leuven, Belgium*

Corresponding author's email: lisanne.groeneveldt@kuleuven.be

The development of alternatives for autologous bone grafts is a major focus of bone tissue engineering. To produce living bone forming implants, skeletal stem and progenitor cells (SSPCs) are envisioned as key ingredients. SSPCs can be obtained from different tissues including bone marrow, adipose tissue, dental pulp and periosteum. Human periosteum-derived cells (hPDCs) exhibit a number of progenitor cell characteristics and have well-documented in vivo bone formation potency. Here, we have characterized and compared hPDCs derived from tibia with craniofacial hPDCs from maxilla and mandible, that could be a potential source for cell-based tissue engineered implants for craniofacial applications.

Maxilla- and mandible-derived hPDCs display similar growth curves as tibial hPDCs, with comparable trilineage differentiation in vitro potential towards chondrogenic, osteogenic and adipogenic cells. These craniofacial hPDCs are positive for the SSPC-markers CD73, CD164 and PDPN (1), and negative for hematopoietic and endothelial lineage markers. Bulk RNA-sequencing identified genes that are differentially expressed between the three sources of hPDCs. In particular, differential expression was found for genes of the HOX and DLX family, SOX9 and genes involved in skeletal system development. De novo in vivo bone formation after ectopic implantation in nude mice was observed in constructs seeded with tibial and mandibular hPDCs.

Taken together, we provide evidence that craniofacial hPDCs are potential sources for cell-based bone tissue engineering strategies. The mandible-derived hPDCs display - both in vitro and in vivo - chondrogenic and osteogenic differentiation potential, which supports their future testing and use for bone regeneration in craniofacial applications.

Keywords

Bone fracture repair; human skeletal stem cell; RNA-sequencing

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Abstract **#1132**

INVESTIGATING EXTRACELLULAR VESICLES FROM HUMAN MESENCHYMAL STEM/STROMAL CELLS AS A THERAPEUTIC APPROACH TO MODULATE IMMUNE RESPONSE

Dimitrios TsiapalisP[1], Tobias Tertel[2], Achilleas Floudas[3], Ursula Fearon[3], Bernd Giebel[2], Verena Boerger[2], Lorraine O'Driscoll[1]

[1] *School of Pharmacy and Pharmaceutical Sciences & Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland*

[2] *Institute for Transfusion Medicine, University Hospital Essen, University of Duisburg-Essen, Essen, Germany*

[3] *Molecular Rheumatology, Trinity College Dublin, Ireland*

Corresponding author's email: TSIAPALD@TCD.IE

Extracellular vesicles (EVs) from human bone-marrow mesenchymal stem/stromal cells (BM-MSCs) are of much interest because they may exert therapeutic effects, such as modulating immune response at a site of injury. However, a reliable potency assay for evaluating immune-modulatory effects of EVs has yet to be established. Towards achieving this, here we isolated and characterised EVs from MSCs cultured under different conditions and assessed their immuno-regulatory properties, in term of influence on inflammatory cytokines release from CD4+ T-cells derived from peripheral blood mononuclear cells (PMBCs) of rheumatoid arthritis (RA) patients.

BM-MSCs were cultured in T175-flasks in media supplemented with either human platelet lysate (hPL) or EV-depleted FBS. After 48h, conditioned media was collected. EVs were isolated using PEG precipitation and characterised using nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), immunoblotting, and flow-imaging (Amnis ImageStream). Flow cytometry (LSRFortessa) analysed a panel of cytokines released from CD4+ T-cells exposed to the MSC-EVs.

NTA indicated significantly more EVs/particles released when MSCs were cultured with hPL-supplemented medium versus medium with EV-depleted FBS. TEM revealed spherical-shaped EVs, with both cultures. Immunoblots showed the presence of EV-associated proteins CD9, CD63, CD81 and Syntenin-1. Supporting these findings, flow-imaging showed increased numbers of CD9+, CD63+ and CD81+ positive sEVs when MSCs were cultured in hPL-medium. Preliminary data demonstrated that the MSC-EVs modulate (typically suppressing) the release of four cytokines that are associated with RA pathogenesis, i.e. TNFα, IL-22, GM-CSF and IL-2, highlighting their immune-regulatory potential. Further studies validating immune-modulatory effects of MSC-EVs on CD4+ T-cells are underway.

Abstract **#1133**

A NOVEL HUMAN SKELETAL MUSCLE IN VITRO MODEL USING OPTI-OX MEDIATED CELLULAR REPROGRAMMING OF INDUCED PLURIPOTENT STEM CELLS

Will Bernard$_P$[1], Imbisaat Geti[1], Esther Walters[1], Patrick Rericha[1], Tüzer Kalkan[1], Michael D'Angelo[1], Giacomo Borsari[1], Dougall Norris[2], Daniel Fazakerley[2], Farah Patell-Socha[1], Thomas Moreau[1], Mark Kotter[1]

[1] *Bit.bio, The Dorothy Hodgkin Building, Babraham Research Campus, Cambridge, CB22 3FH, UK*

[2] *Metabolic Research Laboratories, Wellcome-MRC Institute of Metabolic Science, University of Cambridge, UK*

Corresponding author's email: will.bernard@bit.bio

Skeletal myocytes play roles in a number of biological processes ranging from limb movement to the regulation of nutritional homeostasis, and are implicated in the pathophysiology of a variety of diseases involving muscle dysfunction. There is a pressing need for reliable models of mature human skeletal muscle to permit investigations into physiological and disease mechanisms, and to facilitate the generation of new therapeutics. While human induced pluripotent stem cells (hiPSCs) offer a promising starting material for skeletal muscle cells, their broad use has been hampered by difficult to reproduce, complex differentiation protocols. We have developed an optimised inducible system (opti-ox) that enables tightly controlled expression of transcription factors (TFs) improving cellular reprogramming approaches for the differentiation of hiPSCs. Through targeting of genomic safe harbour loci, we used opti-ox to achieve homogenous, inducible expression of the myogenic regulator MYOD1. MYOD1 induction leads to shutdown of the core pluripotency network, with myogenic factor activation. We demonstrate robust expression of myosin heavy chain, with the transition from immature (MYH3 and MYH8) to mature isoforms (MYH1) expression, in a time dependent manner. opti-ox reprogrammed skeletal myocytes express Desmin, Dystrophin and Titin, and form contractile, striated, multinucleated myotubes. Critically for metabolic studies, robust expression of the insulin-regulated glucose transporter GLUT4 is also detected. The scalability of opti-ox reprogramming provides a unique hiPSC based model of human skeletal myocytes, opening new avenues for high throughput screening and academic research applications, for consistent study of normal and pathological muscle biology.

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Abstract **#1134**

MATURED MYOFIBRES IN BIOPRINTED CONSTRUCTS WITH IN VIVO VASCULARISATION AND INNERVATION IN THE NUDE RAT MODEL

Catherine Ngan[P][1], Anita Quigley[2], Cathal O'Connell[2], Romane Blanchard[1], Richard Williams[3], Tim Aumann[4], Penny McKelvie[5], Gordon Wallace[6], Peter Choong[1], Rob Kapsa[2]

[1] *The University of Melbourne*

[2] *RMIT University*

[3] *Deakin University*

[4] *Florey Institute of Neuroscience and Mental Health*

[5] *Department of Anatomical Pathology*

[6] *University of Wollongong*

Corresponding author's email: cgy.ngan@gmail.com

Introduction: The study of tissue-engineered skeletal muscle has for decades been driven by a clinical need to treat neuromuscular diseases and volumetric muscle loss. The in vitro fabrication of muscle offers the opportunity to test drug and cell-based therapies, to study disease processes, and to perhaps one day serve as a muscle graft for reconstructive surgery. The aim of this study was to develop a biofabrication technique to engineer muscle for research and clinical applications.

Methods: A bioprinting protocol was established to deliver primary mouse myoblasts in a gelatin methacryloyl (GelMA) bioink. Molecular and functional analyses were performed on the bioprinted tissues over two weeks of differentiation. The tissue construct was implanted in an in vivo chamber, supplied by a surgically formed arteriovenous loop and transected nerve in male nude rats (two in both the experimental arm and control groups). After two weeks, the chamber contents were removed for histological analysis.

Results: This work demonstrated the importance of myoblast migration through the GelMA scaffold, with cells spontaneously forming fibres on the surface of the construct. This phenomenon enabled advanced maturation in vitro, and most critically, facilitated the connection between incoming vessels and nerve axons in vivo without the hindrance of a scaffold material. Our preliminary study yielded highly structured, regenerating and developmentally mature myofibres, with early innervation and vascularisation evident within the developing muscle pedicle.

Conclusions: This work developed a bioprinting technique for fabrication of functional skeletal muscle constructs, with applications for both disease modelling and regenerative medicine.

Abstract **#1135**

ANTI-TUMOR EFFECTS OF BIOMIMETIC SULFATED GLYCOSAMINOGLYCANS ON LUNG ADENOCARCINOMA CELLS IN 2D AND 3D IN VITRO MODELS

George Deebp[1], Nada Al Matari[1], Hiba Mshiek[1], Ansam Sinjab[2], Humam Kadara[2], Wassim Abou-Kheir[1], Rami Mhanna[1]

[1] *American University of Beirut*

[2] *University of Texas MD Anderson Cancer Center*

Corresponding author's email: gdd01@mail.aub.edu

Lung adenocarcinoma (LUAD) growth depends on cell proliferation and migration which require interactions with extracellular matrix (ECM) components such as glycosaminoglycans (GAGs). How GAGs regulate cancer cell function is not fully comprehended. A suggested mechanism is it affects the interaction between cancer cells and various growth factors (GF) and cytokines. To better understand how the degree of sulfation (DS) of GAGs affects LUAD cells, sulfated alginates (AlgSulf) with varying degrees of sulfation (DS= 0.0,0.8,2.0,2.7) were synthesized to act as GAG mimics. Human and murine (H1792 and MDA-F471 respectively) LUAD cell lines were treated with AlgSulf of various DSs at two concentrations, 10 and 100 µg/mL. The anti-tumor properties were assessed using MTT, trypan blue exclusion, and wound healing assays for 2D models, while sphere formation assay was used to assess for the 3D model. MDA-F471 cell proliferation and viability decreased significantly at the concentration of 100 µg/mL as the DS of the biomimetic GAGs increased. Cell migration decreased as the DS of biomimetic GAGs increased ($p < 0.001$ for DS =2.0 and 2.7 compared to control) and decreased the diameter and number of spheres formed ($p < 0.001$). Finally, our study shows that GAG-mimetic AlgSulf with an increasing DS exhibits enhanced anti-proliferative and migratory properties and reduces the growth of KRAS-mutant LUAD spheres in vitro. The anti-tumor effects by the GAG-mimetic AlgSulf are possibly due to differential binding of GFs and consequential decreased cell stemness. AlgSulf may prove worthy in cancer therapy after further in vivo validation.

Keywords

Glycosaminoglycans; Lung adenocarcinoma; Sulfated Alginates

Abstract **#1137**

A NEW XENO-HYBRID BONE GRAFT RELEASING MATRIX-DERIVED INTRINSICALLY DISORDERED PROTEINS PROMOTES OSTEOGENIC DIFFERENTIATION OF HMSCS

Håvard J Haugen$_P$[1], Giuseppe Perale[2], Petter Lyngstadaas[1]

[1] *University of Oslo*

[2] *University of Applied Sciences and Arts of Italian Switzerland*

Corresponding author's email: h.j.haugen@odont.uio.no

Bone defect is a significant health problem worldwide. Bone is also the second most transplanted tissue after blood. Myriad bone grafts are designed and applied in clinics. Limitations, however, from different aspects still exist, including limited supply, health complications, mechanical strength, and bioactivity. In this study, two synthetic proline-rich 'disorganized' peptides (P2 and P6) are incorporated into SmartBone® to manufacture a composite bioactive xeno hybrid bone graft named SmartBonePep®, with the aim to increase the bioactivity of the bone graft. The results, which include cytotoxicity, proliferation rate, confocal microscopy, gene expression and protein qualification, successfully prove that the SmartBonePep® has multi-modal biological effects on human mesenchymal stem cells from bone marrow. The successful physical entrapment of P6 into a composite xeno-hybrid bone graft, withstanding the manufacturing processes including exposure to strong organic solvents and ethylene oxide sterilization, increases the osteogenic potential of the stem cells as well as cell attachment and proliferation. P2 and P6 both show a strong biological potential and may be future candidates for enhancing the clinical performance of bone grafts.

Abstract **#1138**

CONTRAST-ENHANCED NANO-CT REVEALS DENTAL SOFT TISSUES AND CELLULAR STRUCTURES

Håvard haugen$_P$[1], Pia Titterud Sunde[1]

[1] *University of Oslo*

Corresponding author's email: h.j.haugen@odont.uio.no

nano-CT technologies offer 3D imaging methods, which allow high-resolution examination of bones and teeth, but soft tissue components have weak X-ray attenuation and are not easily visualized in CT images. We introduce a new methodology designed to simultaneously visualise dental ultrastructure including cellular and soft tissue components, by utilizing PTA in various concentrations and immersion time was tested and scanned with high resolution nano-CT. Three-dimensional nano-CT imaging of dental cementum and periodontium as well as interior components, such as odontoblasts and predentine, with high resolutions was made visible when using PTA staining. The optimal staining protocol differed in different segment of the tooth. The thickness of the cementum could be computed over the height of the tooth made possible by the PTA-enhanced contrast, and the attached soft tissue components of the interior of the tooth could be shown on the dentine-pulp interface in great detail. Three-dimensional illustrations allowed a histology-like visualization of the sections in all orientations with a single scan and easy sample preparation. Furthermore, the dentinal tubules, with the characteristic sigmoid curvature, could be visualized. The developed methodology show that it is possible to visualise hard tissue along with cellular structure and soft tissues using laboratory based nano-CT technique. The staining protocol depended on both tissue type and size. The methodology offers new possibilities for the visualisation of structures at the interphases between soft and hard dental tissue, particularly related to endodontic and periodontal research.

Keywords

nanoCT; imaging cell; Contrast-enhanced nano-CT

Abstract **#1139**

A BIOENGINEERED BONE MARROW NICHE MODEL TO SUPPORT LONG-TERM HSCS IN VITRO

Hannah Donnelly$_P$[1], Ewan Ross[1], Rio Hermantara[2], Christopher West[3], Bruno Peault[3], Adam West[2], Manuel Salmeron-Sanchez[1], Matthew J Dalby[1]

[1] Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, G12 8QQ, United Kingdom.

[2] Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, Glasgow, G61 1QH, United Kingdom.

[3] MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, EH16 4UU, United Kingdom.

Corresponding author's email: hannah.donnelly@glasgow.ac.uk

Hematopoietic stem cells (HSCs) have the ability to regenerate the entire blood and immune system [1], as such, they hold enormous clinical potential. In vivo they are supported by mesenchymal stromal cells (MSCs) and pericytes in a specialised microenvironment that provides physical and functional regulatory cues, termed the bone marrow (BM) niche. Once HSCs are removed from the niche their ability to self-renew is lost quickly, cells rapidly proliferate and spontaneously differentiate away from the long-term reconstituting (LT-HSC) phenotype in a matter of days rendering them clinically ineffective [2]. Here, we have developed a system to recapitulate properties of the BM niche microenvironment to support LT-HSCs in vitro. Using poly (ethyl acylate) (PEA) to promote material-driven unfolding of the extracellular matrix protein fibronectin (FN), we tether BM niche associated growth factors (GF) (e.g. BMP-2) to the exposed GF binding domain on FN [3]. Then by incorporating a collagen type I hydrogel in the stiffness range of the bone marrow (~0.1 kPa) [4], we were able to demonstrate maintenance of a niche-like phenotype in pericytes. This included expression of nestin, a key niche stromal marker [5], and production of HSC maintenance cytokines CXCL12 and SCF. Upon co-culture with HSCs, we found that this niche-like system is able to support LT-HSCs in culture. This material-based system offers a platform for investigation into fundamental mechanisms of stem cell regulation, as well as a more humanised model for genotoxicity screening of drugs, small molecules and cellular therapies.

We acknowledge support of EPSRC grant EP/P001114/1.

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Abstract **#1140**

GUIDED BONE REGENERATION OF CHRONIC NON-CONTAINED BONE DEFECTS USING A VOLUME STABLE POROUS TIO2 SCAFFOLD: AN EXPERIMENTAL IN VIVO STUDY

Minh Khai Le Thieu[P][1], Håvard Haugen[1], Javier Sanz-Esporrin[2], Mariano Sanz[2], Ståle Petter Lyngstadaas[1], Anders Verket[1]

[1] *University of Oslo*

[2] *University Complutense of Madrid*

Corresponding author's email: h.j.haugen@odont.uio.no

Objectives: To evaluate lateral ridge augmentation by guided bone regeneration (GBR) and prospects of space augmentation and maintenance in a chronic non-contained bone defect with the use of a non-resorbable TiO2-scaffold.
Materials and methods: Three buccal bone defects were created in each hemimandible of eight beagle dogs and allowed to heal for eight weeks. Then, treatment by GBR was assigned to following groups by block randomization: Test group: TiO2-scaffold and a collagen membrane, Positive control: Deproteinized bovine bone mineral (DBBM) and a collagen membrane, Negative control: Collagen membrane only. Each hemimandible was randomly allocated to 4 or 12-week healing time. Microcomputed tomography (microCT) was used to measure the width of alveolar bone, graft materials and new bone formation. Histological outcomes included descriptive analysis and histomorphometric measurements.
Results: MicroCT analysis demonstrated increasing new bone formation from four to twelve weeks of healing. The greatest width of mineralized bone was seen in the negative control group, albeit the test group showed the largest ridge volume. The positive control group demonstrated more mineralized bone in the grafted area than the test group, but small amounts were found in both groups. The negative control group presented a natural alveolar ridge shape when not contaminated with graft materials from adjacent sites.
Conclusion: The widest ridge following regeneration was found by the use of a collagen membrane and the non-resorbable TiO2-scaffold. This TiO2-scaffold maintained a larger space but demonstrated less mineralized bone formation than the negative control or the DBBM-graft at the timepoints investigated.

Abstract **#1141**

3D BIOPRINTING A MODEL OF AN EMBRYONIC-LIKE HUMAN HEART TUBE

Jacqueline Bliley[P][1], Andrew Lee[1], TJ Hinton[1], Dan Shiwarski[1], Josh Tashman[1], Adam Feinberg[1,2]

[1] *Department of Biomedical Engineering, Carnegie Mellon University*

[2] *Department of Materials Science and Engineering, Carnegie Mellon University*

Corresponding author's email: jbliley@andrew.cmu.edu

Congenital heart malformations are the leading cause of birth-defect related deaths [1] and occur during early morphogenesis, where the heart starts as a linear tube that bends and loops to transform into its final 4-chambered structure. To date, most of our understanding of heart malformations has been obtained from animal models, but differences in heart physiology and structure in animals when compared to humans limits the value of these models as surrogates for human heart development [2]. Thus, a tissue engineered model of early human heart formation, specifically the linear heart tube, could be tremendously valuable as a tool to expand our understanding of congenital defects. Here, we engineered a bioinspired model of the human heart tube using freeform reversible of embedding of suspended hydrogels (FRESH) 3D bioprinting. The 3D bioprinted heart tubes were cellularized using human stem cell-derived cardiomyocytes and cardiac fibroblasts and formed patent, perfusable constructs. Synchronous contractions were achieved 6 days after fabrication and were maintained for up to a month. Immunofluorescent staining confirmed large interconnected networks of sarcomeric alpha actinin-positive cardiomyocytes. Electrophysiology was assessed using calcium imaging and demonstrated anisotropic calcium wave propagation along the heart tube with a conduction velocity of ~1 cm/s. Contractility and function was demonstrated by tracking the movement of fluorescent beads within the lumen to estimate fluid displacement and force generation. In summary, we have 3D bioprinted a functional embryonic-stage human heart tube, which we plan to later use to model congenital heart defects.

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Abstract **#1143**

INDUCING A LYMPHOID TISSUE ORGANIZER CELL PHENOTYPE IN HUMAN ADIPOSE DERIVED STEM CELLS FOR LYMPHATIC TISSUE ENGINEERING

Jie Gao[P][1], Jan W. Robering[1], Justus P. Beier[1], Anja M. Boos[1]

[1] *Department of Plastic Surgery, Hand Surgery, Burn Center, University Hospital RWTH Aachen, Aachen, Germany.*

Corresponding author's email: jgao@ukaachen.de

Lymphedema is a chronic disease with great impact on the quality of life of patients and is caused by a non-functional lymphatic system. Surgical therapies for the treatment of lymphedema often rely on autologous transplantation of tissue, such as autologous lymph node (LN) transplantation. Despite their advantages compared to decongestion therapy, these techniques can be associated with severe side effects like donor site morbidities. Tissue engineered transplantable LN could help to reduce the need for transplanting autologous tissue as well as the associated side effects. Since LN anatomy is very complex, these organs are difficult to fabricate in vitro. It is therefore useful to mimic the organ's natural development processes in vivo in order to generate transplantable LNs. LN development is governed by the interaction of mesenchymal lymphoid tissue organizer (LTo) cells and hematopoietic lymphoid tissue inducer (LTi) cells. Adipose derived stem cells (ADSC) are similar to LTo in various aspects (morphology, cellular markers). By stimulating ADSC with cytokines involved in the natural development of LN, we tried to induce a LTo-like phenotype in the cells. The expression of CCL19, CCL21 and TRANCE which controls the population of the developing LN by lymphocytes was inducible by lymphotoxin α1β2 and/or TNF-α stimulation. Also the adhesion molecules ICAM1 and VCAM1 that provide adhesion motives for the lymphocytes could be upregulated this way. We demonstrate an in vitro cell culture approach for the conditioning of adult human cell populations by NF-κB activation, which could be applied for the tissue engineering of LN equivalents.

Abstract **#1144**

GET PEPTIDE NANOCOMPLEXES FOR ORAL BARRIER TRANSCYTOSIS AND INSULIN DELIVERY

Sahrish Rehmani[P][1], Kevin Shakesheff[1], James E Dixon[1]

[1] *University of Nottingham*

Corresponding author's email: sahrishrehmani36@gmail.com

Introduction: Oral-insulin-delivery would represent an overwhelming benefit to diabetic patients. Here, we report the use of novel multidomain-peptide system, based on cell-penetrating- peptides, termed Glycosaminoglycan-(GAG)-binding-enhanced-transduction (GET)-system1 as an efficacious transepithelial delivery-vector to promote insulin transcytosis, transduction, and intracellular uptake.

Methods: Fluorescently-labelled-insulin (Ins-F) was complexed with GET to form Ins-F-GET-nanocomplexes (Ins-F-GET-NCs). Caco-2 monolayers (human-colorectal- carcinoma cells) were employed as an in-vitro model for transepithelial delivery, assessing NC stability, and recycling using serial-dosing. NC size and surface-charge was characterised using dynamic-light-scattering (DLS). Insulin-reporter-iLite cells were employed to confirm the functional activity of NCs.

Results: GET-peptides generate NCs (size and charge; 140nm and +27.10mV) that greatly enhance insulin-transport across differentiated in-vitro models of intestinal-epithelium (>22-fold translocation over unmodified-insulin). GET-NCs exhibit continuous post-transfection release from monolayers (both apically and basally) linking uptake and insulin-recycling. Multiple-delivery results in intracellular accumulation, enabling cells to act as depots for sustained insulin-release without affecting cellular-viability and integrity. Importantly both the GET-peptides and Ins-GET-NCs were stable to proteolytic-degradation and stay intact after translocation intracellularly. Functional-assessments using transcription-reporter-assays activated by insulin-signalling revealed that Ins-GET-NCs retain biological activity and can induce pharmacological-response.

Conclusion: GET-NCs promote insulin transcytosis and absorption across in-vitro intestinal model and retain insulin's functional activity. These characteristics are needed for use as a tool for efficacious oral-insulin-delivery. Our system will serve as powerful tool for overcoming the barriers of low permeability of anti-diabetic peptides. Current focus is to improve insulin bio-availability which could allow GET-peptides to be a lead-technology in allowing effective oral-peptide therapeutics for diabetes and beyond.

Keywords

Glycosaminoglycan-(GAG)-binding-enhanced-transduction (GET) system; Oral insulin delivery; Nanoparticle for protein delivery

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Abstract **#1145**

REDUCTION OF CELLULAR MOLECULES OF CORNEAL XENOGRAFTS BY DETERGENT-BASED DECELLULARIZATION SOLUTION AND GAMMA STERILIZATION TO FACILITATE ITS CLINICAL APPLICATION

Miguel Gonzalez-Andrades[P][1,2], Mohammad Mirazul Islam[1], Rakibul Islam[3], Dina B. Abusamra[1], Eleftherios I. Paschalis[1], Per H. Nilsson[3,4], Mario Blanco-Blanco[2], Pablo Argüeso[1], Claes H. Dohlman[1], Tom Eirik Mollnes[3,5,6], James Chodosh[1]

[1] Massachusetts Eye and Ear and Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA.

[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Department of Ophthalmology, Reina Sofia University Hospital and University of Cordoba, Cordoba, Spain.

[3] Department of Immunology, Oslo University Hospital, Rikshospitalet, University of Oslo, Oslo, Norway.

[4] Linnaeus Center for Biomaterials Chemistry, Linnaeus University, Kalmar, Sweden

[5] Research Laboratory, Nordland Hospital, Bodø, and Faculty of Health Sciences, K.G. Jebsen TREC, University of Tromsø, Norway.

[6] Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway.

Corresponding author's email: miguel.gonzalez@imibic.org

Xenografts have the potential to be used as an alternative to human donor corneas but the human immune system hinders their use due to immediate danger sensing by innate immunity and adaptive antibody responses. Here we describe the potential of decellularization and gamma sterilization to reduce the inflammatory response of native porcine corneas (NPC) in human plasma and whole blood.

NPC were decellularized using 0.1% sodium dodecyl sulfate (SDS) and gamma irradiated(1). Collagen type I and antigens associated with acute xenograft rejection (α-Gal and NeuGc) were assessed by western blot (WB). Activation of the human complement system and cytokine release in contact with NPC, decellularized (DPC) and gamma irradiated (G-DPC) porcine corneas, were evaluated using an in vitro model system based on human plasma and blood. One-way ANOVA was used for statistical analysis.

Decellularization removed cellular components from NPC. WB confirmed the preservation of collagen type I ($p=0.1619$). The presence of α-Gal and NeuGc in DPC was significantly reduced compared to NPC ($p=0.0089$ and 0.0454, respectively). The complement activation products C3bc, C4bc, and sC5b-9 showed no significant differences between any of the groups. NPC, DPC and G-DPC induced IL-1β,IL-2,IL-6,IL-8,IL-1ra,MCP-1,MIP-1α,MIP-1β,TNF,G-CSF,INF-γ, and FGF-basic.

Our results confirm that SDS reduces the cellular components and presence of antigens of porcine corneas without altering the native extracellular matrix, which supports cellular regrowth. However, the induced cytokine release should be further investigated to manage its associated in vivo response to facilitate future clinical translation of G-DPC. Partially supported by Andalusian Regional Ministry of Health grant PIGE-0194-2019.

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Abstract **#1146**

SHRINK-WRAPPING CARDIOMYOCYTES IN EXTRACELLULAR MATRIX TOWARDS IMPROVING REPAIR OF DAMAGED HEART MUSCLE

Jacqueline Bliley[P][1], Kristina Kim[1], Rachelle Palchesko[1], Madison Stiefbold[1], Adam Feinberg[1,2]

[1] *Department of Biomedical Engineering, Carnegie Mellon University*

[2] *Department of Materials Science and Engineering, Carnegie Mellon University*

Corresponding author's email: jbliley@andrew.cmu.edu

Heart disease and the associated pathologic changes in the myocardium are difficult to repair because cardiomyocytes, or heart muscle cells, have limited regenerative capacity [1]. This leads to a progressive decline in contractile function and in severe cases, heart failure. Direct injection of cardiomyocytes into the myocardium has been pursued to repair the damaged heart; however, current strategies face many challenges, including low cell engraftment and survival [2]. Here we report a strategy to encapsulate or "shrink-wrap" small clusters of cardiomyocytes within an extracellular matrix (ECM) nano-scaffold to enhance integration into heart muscle. To do this, we use a process we previously developed called surface-initiated assembly [3] to micropattern fibronectin ECM, to which the cardiomyocytes attach, on a sacrificial poly n-isopropyl acrylamide (PIPAAm) substrate. Following PIPAAm dissolution, small clusters of cardiomyocytes are released and the fibronectin ECM effectively shrink wraps around the cluster forming a conformal layer. Proof-of-concept studies performed with C2C12 myoblasts validated that cells could be shrink-wrapped, injected through a needle, and were able to stably integrate into engineered skeletal muscle tissues. Human embryonic stem cell-derived cardiomyocytes were also successfully shrink wrapped and displayed alpha-actinin sarcomeric striations as well as contractile behavior following the shrink-wrapping process. Future work will assess the ability of shrink-wrapped cardiomyocytes to repair damaged heart muscle.

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Abstract **#1147**

SUSTAINED INTRAVITREAL ANTI-VEGF RELEASE FROM PEPTIDE NANOFIBER DELIVERY SYSTEMS

Seher Yaylacı[1]

[1] *Lokman Hekim University*

Corresponding author's email: seherustun@gmail.com

Ranibizumab is a recombinant VEGF-A antibody used in clinics for the treatment of wet form of age-related macular degeneration. It is intraviterally administered to ocular compartments and it needs frequent injections. However, intraviteral administration could cause side complications as well as patient discomfort. These necessitate alternative treatment strategies based on relatively noninvasive ranibizumab delivery that is more effective and sustainable in the eye vitreous than the current clinical regimen. Herein, we developed self-assembled peptide microgels from peptide amphiphile molecules to sustainably release ranibizumab from these microgels at high local dose. Peptide amphiphile molecules, which come together by themselves to form supramolecular filaments, can form gels only in the presence of electrolyte without the need for any agent and provide ease of use compared to polymeric materials. Another great advantage of such systems is that they can be found in free flowing solution as they have low molecular weight and can be easily injected into the targeted area and gel formation can be achieved. Therefore, toxic crosslinkers are not required for the gelation of peptide materials. At the same time, since the peptide materials are completely biodegradable, they do not need to be removed from the injected target when the drug they contain is completely consumed. In this study release profile of ranibizumab at different peptide concentrations was used to evaluate the release performance from the microgels for improved and modulated treatment of wet form of age-related macular degeneration.

Abstract **#1148**

FUNCTIONALISED PEPTIDE HYDROGEL FOR THE DELIVERY OF CARDIAC PROGENITOR CELLS

Kyle Burgess$_P$[1,2], Caterina Frati[3], Kate Meade[1], Jie Gao[1,2], Luis Castillo Diaz[1,2], Denise Madeddu[3], Gallia Graiani[3], Stefano Cavalli[3], Aline Miller[2,4], Delvac Oceandy[5], Federico Quaini[3], Alberto Saiani[1,2]

[1] Department of Materials, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

[2] Manchester Institute of Biotechnology, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

[3] Department of Medicine and Surgery, University of Parma, Italy

[4] Department of Chemical Engineering and Analytical Sciences, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

[5] Division of Cardiovascular Sciences, The University of Manchester, Oxford Road, Manchester M13 9PL UK

Corresponding author's email: kyle.burgess@manchester.ac.uk

Heart failure remains one of the leading causes of death worldwide; typically developing after a myocardial infarction.1 Over 15 years ago, the delivery of cell progenitors was first considered a pioneering strategy to overcome the limited regenerative capacity of the heart; yet to date, such bio-interventions have failed to gain approval for clinical use owing — in part — to poor cell retention and extremely low implantation efficiency.2 As such, there has been a real emergence of biomaterial-based cell delivery strategies for cardiac regeneration.3 In particular, self-assembling peptide hydrogels (SAPHs) offer fully-defined, injectable, biocompatible scaffolds with tuneable properties making them ideal for tissue engineering applications.

We demonstrate that SAPHs (FEFEFKFK) immediately recover following shear stress — using oscillatory rheology — and thereby, remained in the myocardium when injected in vivo. After 7 days, the SAPH (MCA-tagged), plus additional cargo (rhodamine particles), could still be observed within the myocardium. Moreover, SAPHs, both unmodified or modified with the 'RGD' cell-adhesion motif, supported the in vitro culture (3D 'sandwich' model) of cardiac progenitor cells (CPCs). In fact, CPCs recovered from both SAPHs demonstrated a significant propensity to differentiate toward cardiac lineages. Following in vivo delivery, CPCs were retained at the site of injury and observed to differentiate into cardiomyocytes (αSARC+). Injection of SAPH alone temporarily improved cardiac function post-injury but only SAPH + CPCs continued to show improvement beyond 4 days. This work highlights the suitability of SAPHs for cell-delivery and reinforces the need for a biomaterial-based approach for regenerative cardiac therapies.

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Abstract **#1149**

AUTOMATED ACQUISITION AND ANALYSIS PIPELINES FOR (LIVE CELL) MICROSCOPY OF 3D AGGREGATES IN REGENERATIVE MEDICINE

Timo Rademakers[P][1], Clemens van Blitterswijk[1], Vanessa LaPointe[1]

[1] *CBITE, MERLN*

Corresponding author's email: t.rademakers@maastrichtuniversity.nl

Within regenerative medicine, the use of spheroids (3D aggregates) has increased in recent years due to their physiological relevance. To study the structure and function of these spheroids, microscopy is a vital tool, but their size/volume and biological variability can make microscopic imaging daunting. We developed several tools for automated acquisition and analysis, which we used to study how pancreatic spheroids comprising alpha and beta cells respond to local oxygen tension. In a static microwell system, we first used these tools to examine the cell response to oxidative stress. To study the role of local oxygen tension, we then cultured spheroids under flow conditions in a home-built microfluidic chip, in which an oxygen gradient was generated over a microwell array. Automated image acquisition allowed high-throughput detection of individual spheroids, followed by high resolution 3D imaging. During analysis, individual cells were automatically segmented, after which oxidative stress levels could be measured, and morphological data such as spheroid volume and cell distribution within the spheroid were also analysed.

We were able to show how individual cells within spheroids react to oxidative stress to various extent. Moreover, morphological changes within spheroids could be observed upon changes to local oxygen tension. In all, we show the merit of automated microscopy pipelines for both imaging and analysis to increase throughput, improve the reliability of data collected, and reveal new biological insight in cell spheroids.

Abstract **#1150**

ENGINEERING BIOADHESIVE GELATIN-BASED HYDROGEL AS CORNEAL SUBSTITUTE

Miguel Gonzalez-Andrades[P][1,2], Sina Sharifi[2], Mohammad Mirazul Islam[2], Hannah Sharifi[2], Rakibul Islam[3], Darrell Koza[4], Felisa Reyes-Ortega[1], David Alba-Molina[1], Per H. Nilsson[3,5], Claes H. Dohlman[2], Tom Eirik Mollnes[3,6,7], James Chodosh[2]

[1] *Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Department of Ophthalmology, Reina Sofia University Hospital and University of Cordoba, Cordoba, Spain*

[2] *Massachusetts Eye and Ear and Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA*

[3] *Department of Immunology, Oslo University Hospital, Rikshospitalet, University of Oslo, Oslo, Norway*

[4] *Department of Physical Sciences, Eastern Connecticut State University, Willimantic, CT, USA*

[5] *Linnaeus Center for Biomaterials Chemistry, Linnaeus University, Kalmar, Sweden*

[6] *Research Laboratory, Nordland Hospital, Bod, Norway*

[7] *Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway*

Corresponding author's email: miguel.gonzalez@imibic.org

Purpose: Scarcity of donor corneas is the main challenge driving the scientific community to find alternatives for corneal transplantation. Herein, we report the synthesis of a bioadhesive protein-based hydrogel (GELGYM) from grafting functional crosslinkable moieties on gelatin backbone to generate a corneal substitute.

Methods: To synthesize GELGYM with different functionalization degree (FD), a gelatin solution was mixed with different amounts of glycidyl methacrylate (GMA). The reaction mixtures were dialyzed and freeze-dried to obtain foam-like GELGYM precursors. These were dissolved in varying amounts of PBS to form different concentrations of GELGYM, and mixed with crosslinking solution (eosin Y, vinyl caprolactam, and triethanolamine). The resulting solution was carefully transferred to an appropriate mold or applied to the desired structure and light-crosslinked for various times (1-10 min). Chemical, mechanical, and optical properties, and biocompatibility, of the engineered hydrogel were analyzed.

Results: Chemical characterizations demonstrated the formation of GELGYM with different FD, depending on the reaction conditions. GELGYM can be stretched up to 4 times its initial length and can withstand high tensile stresses up to 1.95 MPa with compressive strains as high as 80% without breaking, as indicated by tensile and compression tests. GELGYM was highly transparent, adhesive and biocompatible, supporting cellular proliferation and migration in both 2 and 3-dimentional cell-cultures, without affecting corneal cell phenotypes.

Conclusions: GELGYM has tuneable structural and biodegradative characteristics, which can be controlled by varying FD, crosslinking time, and prepolymer concentration. These, along with its biocompatibility and adhesiveness suggest its application as corneal stromal substitute or filler.

Abstract **#1151**

USE OF IN SILICO MODELLING FOR QUANTIFICATION OF MECHANICAL LOADING OF CELLS AND NOZZLE SHAPE OPTIMISATION IN 3D EXTRUSION BASED BIOPRINTING

Sourav Mandal[1], Esther Reina-Romo[2], Nina Van Steenvoort[3], Paulo Amorim[4,5], Veerle Bloemen[4,5], Eleonora Ferraris[6], Liesbet Geris[1,3,4]

[1] *Biomechanics Research Unit, GIGA In silico Medicine, Université de Liège (Belgium)*

[2] *University of Seville. Department of Mechanical Engineering and Manufacturing. Seville (Spain)*

[3] *Biomechanics Section, Department of Mechanical Engineering, KU Leuven (Belgium)*

[4] *Prometheus, the Division of Skeletal Tissue Engineering, KU Leuven (Belgium)*

[5] *Materials technology TC, Campus Group T, KU Leuven (Belgium)*

[6] *Department of Mechanical Engineering, Campus de Nayer, KU Leuven (Belgium)*

Corresponding author's email: mandalsouravr@gmail.com

3D bioprinting is a flourishing fabrication technology in regenerative medicine, whose potential large-scale implementation is hindered by technical hurdles such as printability and cell-survivability along with the time and resource-intensive in vitro screening experiments, employed for every new printing condition. We sought to provide the appropriate choice of a nozzle design base on shear stress, well-known as a crucial factor for cell-survivability in extrusion-based 3D bioprinting. Three widely used shear-thinning hydrogel materials in two different nozzle configurations were tested. Nozzle design parameters were tested in the range relevant to practical application, using Latin hypercube sampling (LHS). Computational fluid dynamics (CFD) simulations were performed to quantify the flow profile and the resulting shear stresses. The outcome of CFD from the different combinations was fitted into a machine learning method, 'Gaussian process' (GP). The relationship of each nozzle design parameter and its relative importance on the response was obtained from the GP. This suggested that both material properties (power-law index) and the lower nozzle length and exit radius are the most important parameters for blunted nozzles, whereas the middle radius of the conical nozzle is an additional crucial factor influencing shear stress. The importance of the current approach lies in eliminating non-influential variables and providing a quantitative description of influential ones using a broad range screening that would be almost impossible using conventional in vitro experiments. In summary, we demonstrate the efficacy of in silico modelling as the feasible alternative to lower the burden of costly experimental trial-and-error and aid future development.

Abstract **#1152**

BIOFABRICATION OF ARTIFICIAL TRACHEA AND ESOPHAGUS USING KENZAN METHOD

Keitaro Matsumoto[P][1,2], Ryusuke Machino[1,2], Daisuke Taniguchi[1,2], Yosuke Takeoka[1,2], Shozaburo Oyama[1,2], Tomoyuki Tetsuo[1,2], Masaaki Moriyama[1,2], Fumitake Uchida[1,2], Ryosuke Hara[1,2], Kaido Oishi[1,2], Yasuaki Taura[1,2], Katsunori Takagi[1,2], Tomoshi Tsuchiya[1], Koichi Nakayama[3], Takeshi Nagayasu[1,2]

[1] *Department of Surgical Oncology, Nagasaki University*

[2] *Medical-engineering hybrid professional development center, Nagasaki University*

[3] *BioMedical Engineering Course Advanced Technology Fusion Graduate School of Science and Engineering, Saga University*

Corresponding author's email: kmatsumo@nagasaki-u.ac.jp

Introduction; Most artificial organs with scaffolds have some limitations, such as inflexibility in design, lack of cytocompatibility, toxic effects, and post-transplant degradation. The present study sought to generate scaffold-free trachea and esophagus-like structures using several types of human cells by a bio-three-dimensional (3D) printing system. The rationale of the present study was to generate a scaffold-free trachea esophageal-like structure using bio-three-dimensional (bio-3D) printing technology and to assess circumferential replacement using the structure. Methods; human cartilage cells (NHACs), human fibroblasts (NHDFs), human umbilical vein endothelial cells (HUVECs), human esophageal smooth muscle cell (hESMCs) and human mesenchymal stem cells from bone marrow (MSCs) were used. By using bio-3D printer "Regenova®", structures with different combinations of cells were made for trachea and esophagus. The structures were transplanted into F344 rats with immunosuppressant. Results; We transplanted the structures of ladder shape for trachea and simple structure for esophagus. We replaced the trachea by the structure circumferentially and for esophagus performed end to side anastomosis to abdominal esophagus and stomach. The rats survived one month and more after surgery. The structures were matured in vivo with epithelial cells extended from native trachea and esophagus and with micro vessels and blood cell. Conclusions; we will show the possibility of artificial organs made by bio-3D printer.

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Abstract **#1153**

THE INTERNATIONAL SPACE STATION NATIONAL LAB – STEM CELLS AND BIOFABRICATION

Marc Giulianotti[1], Michael Roberts[1]
[1] *Center for the Advancement of Science in Space*
Corresponding author's email: mgiulianotti@issnationallab.org

The International Space Station (ISS) U.S. National Laboratory is enabling a new era of research in space aimed at improving life on Earth. The ISS National Lab accomplishes this by promoting and brokering a diverse range of research in life sciences, physical sciences, remote sensing, technology development, and education.

The ISS National Lab has a strong record of supporting stem cell research and biofabrication in space. These efforts have been enabled through the use of direct solicitations, partnerships with other government agencies, and collaborations between commercial companies and foundations. The talk will outline some of the projects and programs developed through these efforts and highlight some of the key findings from the studies. Additionally, we will overview current opportunities for the research community.

As we enter the third decade of continuous human occupancy of the ISS we continue to expand the use of the ISS National Lab. One area of expansion comes in developing public-private partnerships aimed at enabling in space biomanufacturing. While the focus of the ISS National Lab is to encourage these partnerships for the development of technologies that directly impact humans on Earth there are clear overlaps for exploration efforts. The talk will conclude with a summary of where these efforts currently stand and overview some of the planned next steps.

Abstract **#1154**

CONTROL OF STAPHYLOCOCCAL-MEDIATED PROTEASE ACTIVITY USING CELLULOSE-COLLAGEN WOUND DRESSINGS IN A DIABETIC FOOT MODEL

Rahaf Issa$_P$[1], Kate Thompson[1], Bianca Price[1]

[1] *Department of Pharmacy and Optometry, The University of Manchester, Manchester, UK.*

Corresponding author's email: rahaf.issa@manchester.ac.uk

Chronic wounds, such as diabetic foot ulcers, are maintained in a state of constant inflammation, characterised by abundant levels of proteases, which can delay wound healing. Selecting a dressing capable of controlling such factors can have a significant impact on the healing of chronic wounds. In this study, we developed an in vitro complex chronic wound model, consisting of fibroblasts and keratinocytes in a collagen matrix, to assess the effects of two oxidised regenerated cellulose (ORC)/collagen dressings, Promogran® and Promogran Prisma®. Models were wounded using a biopsy punch. Cells and models were maintained in a persistent state of inflammation using 5-10% Staphylococcal supernatant. In keratinocyte scratch assays, exposure to Staphylococcal supernatant increased protease production by 6.3-fold and delayed cell migration without affecting cell viability; leaving the scratch area 60% open after 48hrs. In the models, unstimulated wounds showed signs of healing within 6 days of wounding, and resembled unwounded skin by day 10. However, stimulation with Staphylococcal supernatant delayed wound healing, with no evidence of infiltrating fibroblasts or keratinocytes at day 6. Both dressings reduced protease levels in stimulated models within two days of treatment. Protease levels returned to baseline within 10 days of treatment with Promogran®, and 6 days of treatment with Promogran Prisma®. Stimulated wounds, that previously did not show any signs of healing, fully healed within 6 days of treatment with the dressings. In summary, ORC/collagen dressings mitigate the negative effects of Staphylococcal-mediated protease activity on wound healing in our in vitro model.

Keywords

Wound healing; Protease activity; Dressings

Abstract **#1155**

DEVELOPMENT OF A DECELLULARIZED ECM BIOINK FOR 3D BIOPRINTING OF HUMAN SKIN EQUIVALENTS

Atiya Sarmin[P][1], Ratima Suntornnond[2], Nadia El Moussaid[1], Jonathan Dawson[3], John Connelly[1,2]

[1] *Blizard Institute, Queen Mary University of London, UK*

[2] *CREATE Lab, Blizard Institute, Queen Mary University of London, UK*

[3] *Faculty of Medicine, University of Southampton, UK*

Corresponding author's email: a.m.sarmin@qmul.ac.uk

Skin is a highly complex, hierarchical, and dynamic organ that consists of an epidermal barrier layer and dermal matrix, containing hair follicles, glands, blood vessels, and nerves. The current 3D organotypic co-cultures are simplistic models that fail to capture many of the key biological functions of human skin and are limited in their ability to model normal tissue function and disease processes. This project aims to develop next-generation in vitro models of human skin using 3D bioprinting to mimic the tissue's essential structural and cellular complexity.

To establish an appropriate bioink to be used in the human skin equivalent model, we have purified decellularised ECM (dECM) from porcine skin. Western blot and Mass Spectometry analyses demonstrated the success of the dECM protocol by indicating the presence of major ECM molecules including, collagen I and fibrillin. Rheological analyses demonstrated the shear thinning properties of dECM hydrogels, indicating its suitability as a bioink. When used as a dermal matrix for 3D skin equivalent models, dECM hydrogels supported epidermal stratification with expression of terminal differentiation markers and the presence of a defined cornified layer. Finally, the dECM was compatible with advanced fabrication methods, including the use of sacrificial materials and printing within a gelatin support bath, thereby demonstrating its flexibility as a bioink.

Thus, the dECM hydrogel developed here represents a promising biomaterial for 3D bioprinting, and future studies will apply this bioink to the development of next-generation skin equivalent models.

Abstract **#1156**

IN VIVO MODULATION OF MSC-DIRECTED HOST IMMUNE RESPONSE BY SI-HPMC HYDROGEL

Noelle MATHIEU$_P$[1]

[1] *IRSN*

Corresponding author's email: noelle.mathieu@irsn.fr

MSC therapy is considered as promising option to treat various disorders. Based on hundreds of clinical trials the safety of MSC therapy seems clear. However, they do not confer expected beneficial effect, suggesting a real need to accelerate research towards the successful clinical application. We previously showed that embedding MSC from rat in a hydrogel, namely Silanized HPMC, increased the number of engrafted cells and MSC can be detected until 21 days in irradiated rat colonic mucosa [1-2]. However, biomaterials, according to their composition influence the biocompatibility with the host organism and the inflammatory and immune reaction. In the present study, we analysed in vivo the MSC-directed host immune response when embedded in Si-HPMC hydrogel.

We demonstrated that Si-HPMC hydrogel and Si-HPMC embedded hu-MSC injections do not induce statistical increase of innate immune infiltrate. Adaptive immune response has also been evaluated by measuring (i) serum antibodies directed specifically against hu-MSC and (ii) apoptosis of hu-MSC induced by cytotoxic lymphocytes. We demonstrated that the level of anti-huMSC antibodies and hu-MSC apoptosis induced by lymphocytes are statistically reduced when the cells are embedded in Si-HPMC compare with cells resuspended in PBS1x.

Altogether these data demonstrated that embedding MSC in Si-HPMC decrease the specific immune response directed against xenogenic MSC and favour MSC survival in the inflamed tissue that could explain the increase their therapeutic benefit. This also open perspectives for allogenic clinical use.

Keywords

Mesenchymal stromal cells

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Abstract **#1159**

MASS SPECTROMETRY IMAGING AND PROFILING OF SINGLE CELLS: APPLICATION IN BREAST CANCER RESEARCH

Eva Cuypers[P][1], Rianne Biemans[2], Natasja Lieuwes[2], Ludwig Dubois[2], Ron M.A. Heeren[1]

[1] *M4I Institute, Division of Imaging Mass Spectrometry, University Maastricht, The Netherlands*

[2] *The M-Lab, Department of Precision Medicine, University Maastricht, The Netherlands*

Corresponding author's email: e.cuypers@maastrichtuniversity.nl

Introduction

Single cell technologies in cancer research are recently strongly developing since these enhance the molecular understanding. Biomolecules such as lipids are known to play a major role in cancer biology. Being able to visualize their distribution on a single cell level will open up new molecular information on the development, growth and heterogeneous composition of cancer cells. We present a single-cell imaging mass spectrometry method as powerful tool to map biomolecule distribution.

Material & Methods

Breast cancer cell lines were cultured, plated onto coated ITO slides and incubated overnight. Washing and drying steps were applied. Prior to imaging, norhamane matrix was sublimed onto the cell containing slide. Single cell chemical profiling was achieved using 5 µm spatial resolution MALDI imaging coupled to high mass resolution detectors (FTICR & TimsTOF). SCiLS Lab software (Bruker) was used for visualization and statistical analysis.

Results

The full chemical composition of breast cancer cell lines, including lipids identification, is generated on a single cell level. Thanks to the applied imaging technique, lipid distributions within a single cell are visualized. Based on statistical analysis of the single cell profiles, we generated a model that is able to distinguish different cell types.

Conclusion

Our direct identification and visualization method of biomolecules on a single cell level opens new possibilities in breast cancer research. Cellular differences can be detected, possibly leading to the identification of specific molecular pathways and biomarkers. Moreover, visualization of compound distributions within a single cell is a major step in understanding intracellular pathways.

Keywords

Imaging Mass Spectrometry; Single cell profiling; Breast cancer

Abstract **#1160**

MESENCHYMAL STEM CELLS SECRETOME IN PARKINSON'S DISEASE REGENERATIVE MEDICINE: USING BRAINS ORGANOIDS AS A TOOL

Bárbara Mendes Mendes-Pinheiro[P][1,2], Sarah Nickels[3], Ibrahim Boussaad[3], Rejko Krueger[3], António J. Salgado[1,2], Jens C. Schwamborn[3]

[1] *Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho*

[2] *ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal*

[3] *Luxembourg Centre for Systems and Biomedicine (LCSB), University of Luxembourg (UL)*

Corresponding author's email: barbarafmpinheiro@gmail.com

Parkinson's disease (PD) is an age-related neurodegenerative disorder, characterized by a selective loss of dopamine (DA) neurons causing motor dysfunctions1. The exact mechanism behind dopaminergic cell death is still unknown, being therefore a challenge the development of reliable disease models that can truly recapitulate the features of the disease. Recent advances have prompted the development of human brain organoid systems, that mimic the organization and function of brain regions2, which in turn can be used as platforms for studying the neuroprotective effects of newly therapeutic tools, like the secretome of mesenchymal stem cells (MSCs). Thus, the first objective of this work was to modulate neuronal degeneration in human midbrain organoids (hMOs), to foster dopaminergic repair, using the secretome of bone marrow-derived MSCs. For that, two different in vitro PD models were used: hMOS treated with 6-OHDA to induce DA neurons degeneration, and hMOS carrying DJ-1 mutation (known to cause autosomal recessive PD). The hMOS generation was based in the protocol developed by Monzel et al.2, and in both models the cultures were exposed to MSCs secretome. Through high-content image analysis we observed that treated hMOS had more TH-positive cells and less fragmented neurons when compared to the untreated cultures, in both models. Considering these results, we are currently developing a combined PD mice model, using DJ-1 KO animals exposed to 6-OHDA. A combined may provide new insights from the point of view of the dual-hit hypothesis, representing a more robust model to study the therapeutic effects of MSCs secretome.

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Abstract **#1161**

DESIGN AND DEVELOPMENT OF A REINFORCED TUBULAR ELECTROSPUN CONSTRUCT FOR THE REPAIR OF RUPTURES OF DEEP FLEXOR TENDONS

Nele Pien[P][1,2], Ian Peeters[3], Liesbet Deconinck[1], Lana Van Damme[1], Lieven De Wilde[3], Ann Martens[4], Sandra Van Vlierberghe[1], Arn Mignon[1], Peter Dubruel[1]

[1] Polymer Chemistry & Biomaterials Research Group, Centre of Macromolecular Chemistry (CMaC), Ghent University, Krijgslaan 281, S4 bis, 9000 Gent, Belgium,

[2] Laboratory for Biomaterials and Bioengineering, Department of Min-Met-Materials Engineering & Regenerative Medicine, CHU de Quebec Research Center, Laval University, 2325 Rue de l'Université, Québec G1V 0A6, Canada,

[3] Faculty of Medicine and Health Sciences, Department of Human Structure and Repair, Ghent University Hospital, C. Heymanslaan 10, ingang 46, 9000 Gent, Belgium,

[4] Faculty of Medicine and Health Sciences, Department of Surgery and Anaesthesiology of Domestic Animals, Ghent University Hospital, Belgium

Corresponding author's email: nele.pien@ugent.be

The repair of traumatic and degenerative ruptures of deep flexor tendons remains one of the most intriguing topics in hand surgery. Current treatment methods fail in efficient repair of the injured tendon, and are mainly challenged in attaining sufficient mechanical strength and in limiting adhesion and inflammation issues. This research aims at developing a more potent solution for deep flexor tendon repair by combining a mechanical (improved mechanical properties) and biological approach (incorporation of bio-active compounds). A reinforced, multi-layered electrospun tubular construct is developed, composed of three layers: an inner electrospun layer containing an anti-inflammatory component (Naproxen), a middle layer of braided monofilament as reinforcement and an outer electrospun layer containing an anti-adhesion component (hyaluronic acid, HA).

In a first step, a novel acrylate endcapped urethane-based precursor (AUP) is developed and characterized by measuring molar mass, acrylate content and thermo-stability. The AUP material is benchmarked against commercially available poly(ε-caprolactone) (PCL). Next, the materials are processed into multi-layered, tubular constructs with bio-active components (Naproxen and HA) using electrospinning. In vitro assays using human fibroblasts show that incorporation of the bio-active components is successful and not-cytotoxic. Moreover, tensile testing using ex vivo sheep tendons prove that the developed multi-layered constructs fulfill the required strength for tendon repair (i.e. 2.79 - 3.98 MPa) [1], with an ultimate strength of 8.56 ± 1.92 MPa. In conclusion, by combining a mechanical approach with the incorporation of bio-active compounds (biological approach), this solution shows its potential for application in deep flexor tendon repair.

Keywords

Tendon Repair; Electrospinning; Reinforced construct

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Abstract **#1162**

TREATMENT OF HEPATIC STEATOSIS: THE POTENTIAL OF NANOGEL-MEDIATED DELIVERY OF HYDROXYTYROSOL

Emanuele Mauri[1], Manuele Gori[1], Sara Maria Giannitelli[1], Andrea Zancla[1], Pamela Mozetic[2], Nicolò Merendino[3], Filippo Rossi[4], Marcella Trombetta[1], Alberto Rainer[1,2]

[1] Department of Engineering, Università Campus Bio-Medico di Roma, Rome (Italy)

[2] Institute of Nanotechnologies (NANOTEC), National Research Council, Lecce (Italy)

[3] Department of Ecological and Biological Sciences, Università degli Studi della Tuscia, Viterbo (Italy)

[4] Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan (Italy)

Corresponding author's email: s.giannitelli@unicampus.it

Nonalcoholic fatty liver disease (NAFLD) is considered a leading cause of chronic liver disease worldwide and it encompasses the simple steatosis to the more severe steatohepatitis, including cirrhosis and hepatocellular carcinoma[1-2]. Although no effective treatments are available, hydroxytyrosol (HT) has been shown to contrast the development of hepatic steatosis through its lipid-lowering, antioxidant and anti-inflammatory activities. However, the efficient delivery of this molecule to hepatocytes remains a crucial aspect: we propose the design of smart traceable nanogels as a promising tool to promote the intracellular HT release, avoiding metabolic alterations and the loss of the active molecule in the extracellular environment. Nanogels are composed by polyethylene glycol and rhodamine-labeled polyethyleneimine[3], and their performance was evaluated in an in vitro model of hepatic steatosis characterized by a fat overload induced through a mix of free fatty acids (FFA)[4]. The obtained results show a substantial decrease in the intracellular triglyceride accumulation only when HT was delivered by the nanovectors, improving the efficacy with regard to the current administration of HT in its free form. Moreover, oxidative stress levels were not detected in the treated cells and the reduced cell viability associated to FFA overload was significantly mitigated using the nanogel-delivered HT, restoring to a healthy liver condition. Hence, these formulated nanogels may represent a more effective strategy compared to common drug administration routes for protecting liver cells from the development of hepatic steatosis.

Keywords

Nanogels; Polyphenols; Hepatic steatosis

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Abstract **#1163**

SYNTHETIC EXTRACELLULAR MATRICES BASED ON SUPRAMOLECULAR MATERIALS

Patricia Dankers$_P$[1]

[1] *Eindhoven University of Technology*

Corresponding author's email: p.y.w.dankers@tue.nl

The extracellular matrix (ECM) is a complex assembly of various molecules held together via both covalent and noncovalent interactions. In order to make materials with comparable properties it is proposed that supramolecular materials based on hydrogen bonding units are eminently suitable. Synthetic supramolecular materials can be made that are composed of supramolecular monomers polymerized via similar interactions. This results in dynamic materials. An important challenge in the synthesis and formulation of synthetic extracellular matrices, is besides the balance between dynamics and robustness, the introduction of complexity. This complexity can exist of bioactive molecules that are either supramolecularly or covalently attached to the materials, or might originate from the assembly of the supramolecular monomers into various (hierarchical) structures. Both parameters will heavily influence the function of the materials synthesized when brought into contact with cells and tissues. The bioactivity we have introduced into our supramolecular systems is based on small synthetic peptides, large ECM proteins, carbohydrates or decellularized ECM. In this way we studied the complexity of the bioactive signals displayed to the cells. Interestingly, small synthetic peptides can outperform mixtures of a large amount of different bioactive compounds, highlighting the importance of simple synthetic systems.

Abstract **#1164**

A NEUROVASCULAR 3D CELL MODEL TO INVESTIGATE THE ROLE OF PERICYTES IN DEMENTIA

Marlene Polleres$_P$[1], Geoffrey Potjewyd[1], Tao Wang[2], Marco Domingos[3,4], Nigel Hooper[1]

[1] *Division of Neuroscience & Experimental Psychology, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, UK*

[2] *Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, UK*

[3] *Department of Mechanical, Aerospace and Civil Engineering, School of Engineering, Faculty of Science and Engineering, The University of Manchester, UK*

[4] *The Henry Royce Institute, Alan Turing Building, Oxford Road, Manchester, M13 9PL, UK*

Corresponding author's email: marlene.polleres@manchester.ac.uk

Pericytes, brain microvascular endothelial cells (BMECs), astrocytes, and neurons form the neurovascular unit (NVU). NVU dysfunction is commonly seen in vascular dementia and Alzheimer's disease (1). Pericytes have been shown to contract capillaries and thus reduce cerebral blood flow, in a downstream response to amyloid-β, a hallmark protein of Alzheimer's disease (2). However, the full role of pericytes in health and disease remain poorly understood, partially due to a lack of adequate models that can recapitulate the complexity of the multi-cellular NVU. The aim of this study is to incorporate pericytes into a 3D in vitro model to study the function, and dysfunction, of the NVU and the pericytes within it. Brain pericyte-like cells and BMECs were differentiated from human induced pluripotent stem cells (iPSCs) (3, 4) and co-cultured in a transwell-type NVU model. Integrity of the endothelial barrier function was evaluated by transendothelial electrical resistance (TEER) measurements. Hydrogel encapsulation was used as a contraction indicator. Brain pericyte-like cells stained positively for pericyte markers NG2, PDGFR-β, CD13, and CD146. Co-culture of pericyte-like cells with BMECs resulted in a significantly increased TEER compared with mono-cultured BMECs. Furthermore, these brain pericyte-like cells significantly decreased the hydrogel surface area by 40% showing their potential ability to contract. These results suggest that functional brain pericyte-like cells were obtained and can be incorporated into a NVU model. We will build on these findings to develop a robust model to study pericytes within the NVU to investigate their function and dysfunction in health and disease.

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Abstract **#1167**

ENGINEERED MICROENVIRONMENTS FOR SYNERGISTIC TGFBR-INTEGRIN CROSSTALK DRIVEN CHONDROGENESIS OF PATIENT DERIVED PERICYTES FOR MICROTIA REPAIR

Hannah Donnelly[P][1], Alina Kurjan[1], Li Yong[2], Yinbo Xiao[1], Christopher West[2], Manuel Salmeron-Sanchez[1], Matthew J Dalby[1]

[1] *Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, G12 8QQ, United Kingdom.*

[2] *MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, EH16 4UU, United Kingdom.*

Corresponding author's email: hannah.donnelly@glasgow.ac.uk

Microtia is a congenital deformity of the external ear that ranges from complex craniofacial deformities where ears are virtually absent, to ones that are correctly formed but smaller than usual[1]. This condition affects 1 in 6,000 infants born in the UK annually[1]. Currently, microtia patents are limited in their therapeutic options. Typically, bone marrow mesenchymal stromal cells (MSCs) have been investigated as a cell source for generation of cartilaginous grafts[2]. Standard induction of chondrogenesis uses high density culture (HDC) and chondrogenic media (CM). CM typically contains a cocktail of dexamethasone and transforming growth factor-beta 1 (TGFb1). However, dexamethasone is also a major component of osteogenic media[3]. Leading to off-target effects such as the proclivity of MSCs to undergo hypertrophic differentiation[4]. Here, we demonstrate isolation of a novel cell source; CD146+ pericytes from microtia biopsies vasculature (ear pericytes; EPs) and culture in a chondrogenic-targeting microenvironment. Using poly (ethyl acylate) (PEA) to promote material-driven unfolding of the extracellular matrix protein fibronectin (FN), we tether TGFb1 to the exposed GF binding domain on FN[5]. The PEA system permits targeted delivery of nano-doses of GFs to cells, potentially reducing off-target effects associated with complex CM formulations[5]. We demonstrate that recapitulating aspects of the native niche microenvironment using the EPs seeded in HDC onto the PEA system leads to chondrogenesis that does not exhibit the hallmarks of hypertrophy. As PEA can coat 3D structures, it's use is easily envisioned this technology could be used on complex human ear shaped scaffolds.

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Abstract **#1168**

NEURAL PRECURSORS CELLS EXPANDED IN A 3D MICRO-ENGINEERED NICHE PRESENT ENHANCED THERAPEUTIC EFFICACY IN VIVO

Stephana Carelli[P][1], Toniella Giallongo[2], Federica Rey[2], Bianca Barzaghini[3], Tommaso Zandrini[4], Andrea Pulcinelli[3], Riccardo Nardomarino[3], Giulio Cerullo[4], Roberto Osellame[4], Cristina Cereda[5], Gian Vincenzo Zuccotti[1], Manuela Teresa Raimondi[3]

[1] *Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", L. Sacco Department of Biomedical and Clinical Science, University of Milano, Milano, Italy*

2 University of Milan

3 Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy

4 Istituto di Fotonica e Nanotecnologie (IFN)-CNR and Department of Physics, Politecnico di Milano, Milano, Italy

5 Genomic and Postgenomic Lab, IRCCS Mondino Foundation, Pavia, 27100, Italy

Corresponding author's email: stephana.carelli@unimi.it

INTRODUCTION: Biomaterials have been used to create micro-scaffolds, allowing the generation of active biophysical signals for directing stem cell fate. The Nichoid, has been reported to maintain the stemness capacity in cells of different origins [1]. The aim of this study was to investigate: i) the proliferation, differentiation and stemness properties of neural precursor cells expanded inside the Nichoid (NIC-NPCs); ii) the therapeutic potential of NIC-NPCs in preclinical experimental model of Parkinson's Disease (PD) [2,3]. METHODS: Nichoids were fabricated by two-photon laser polymerization using an organic-inorganic photoresist. NPCs expanded inside the Nichoid were counted and analyzed through RNA-seq, Real Time PCR, western blot and immunofluorescence. Parkinsonism was induced by the administration of MPTP in C57/black[3] and the recovery of function was investigated with behavioral tests. RESULTS: 7 days after plating, the NIC-NPCs show a significantly higher proliferation and viability than in standard floating conditions and NIC-NPCs show an increase in pluripotency. Moreover, NIC-NPCs showed a potentiation in their therapeutic features by counteracting neurodegeneration and promoting recovery of function in vivo. These evidences are supported by histological analysis of specific molecular markers. Moreover, NIC-NPCs downregulated gliosis. CONCLUSIONS: Here we provide evidences that NIC-NPCs both favors their pluripotency and potentiates their efficacy showing great promise in the field of regenerative medicine.ACKNOWLEDGEMENTS: Financial support was received from "Neurogel-en-Marche" Foundation; Fondazione "Romeo and Enrica Invernizzi"; ERC grant NICHOID, G.A. 646990 and NICHOIDS, G.A. 754467.

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Abstract **#1170**

MICRORNA CARGO OF UMBILICAL CORD-DERIVED EXTRACELLULAR VESICLES AND ITS POTENTIAL ROLE IN NEUROREGENERATION

Marianne Joerger-Messerli[1,2], Valérie Haesler[1,2], Gierin Thomi[1,2], Irene Keller[1,3], Daniel Surbek[1,2], Andreina Schoeberlein[1,2]

[1] *Department for BioMedical Research (DBMR), University of Bern, Switzerland*

[2] *Department of Obstetrics and Feto-maternal Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland*

[3] *Swiss Institute of Bioinformatics, University of Bern, Switzerland*

Corresponding author's email: marianne.joerger@dbmr.unibe.ch

Mesenchymal stromal cells (MSC) derived from the umbilical cord connective tissue, the so-called Wharton's Jelly (WJ) have a promising regenerative potential, which is mainly attributed to the cells' secretome including extracellular vesicles (EV). EV carry functional microRNAs (miRNAs), which are delivered to target cells.

We hypothesize that WJ-MSC-derived EV contain miRNAs, that interfere with signaling pathways involved in brain injury of premature infants. Thus, we aimed to characterize and quantify the miRNA content of WJ-MSC-derived EV.

EV were retrieved from WJ-MSC culture supernatants by serial highspeed and ultracentrifugation. A genome-wide profiling of paired WJ-MSC and WJ-MSC-derived EV from the same individuals (n=6) was done by preparing small RNA libraries, and Illumina NextSeq Sequencing. KEGG pathway enrichment analysis was done by DIANA-miRPath v.3.

In EV, 850 miRNAs were expressed (mean normalized counts > 1). Thereof 213 were significantly upregulated in WJ-MSC-derived EV compared to their donor cells, while 120 were downregulated. KEGG pathway enrichment analysis showed that the miRNA present in WJ-MSC-derived EV targeted genes having key roles in pathways related to apoptosis, neuroinflammation and the maturation of the oligodendrocyte lineage.

Our data strongly suggest that several miRNAs found in WJ-MSC-derived EV have key functions in neuroregeneration, substantiating their therapeutic potential in premature brain injury.

Abstract **#1171**

THE ROLE OF HOFFA'S FAT PAD IN ASSESSING THE REGENERATIVE CAPACITY OF THE CARTILAGE IN OSTEOARTHRITIS AND CARTILAGE DEFECT PATIENTS.

Mirella Haartmans[P][1,2], Britt Claes[1], Maxime Eveque-Mourroux[1], Gert Eijkel[1], Kaj Emanuel[2], Gabrielle Tuijthof[3], Lodewijk van Rhijn[2], Ron Heeren[1], Pieter Emans[2], Berta Cillero-Pastor[1]

[1] Maastricht Multimodal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, the Netherlands

[2] Laboratory for Experimental Orthopedics, Department of Orthopedic Surgery, CAPHRI Care and Public Health Research Institute, Maastricht University Medical Center+, Maastricht, the Netherlands

[3] Department of Instrument Development Engineering & Evaluation (IDEE), Maastricht University, Maastricht, the Netherlands

Corresponding author's email: m.haartmans@maastrichtuniversity.nl

Background: Knee osteoarthritis (OA) is a degenerative joint disease affecting the articular cartilage and its surrounding tissues. OA development is often associated with cartilage damage earlier in life and appears to be increasing in the younger population. Sufficient treatment of osteochondral defects and early detection of OA are crucial for possible cartilage regeneration and postponement of OA development. In this work we studied the lipid signature of Hoffa's fat pad (HFP) of patients with OA or a cartilage defect (CD) using Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), in order to characterize novel biomarkers for OA and cartilage regeneration.

Methods: Snap-frozen HFP of three OA and three CD patients were cryosectioned at 15 µm and sublimed with Norharmane matrix before being analyzed using MALDI-MSI (negative and positive ion modes) on a RapifleX Tissuetyper at 50 µm lateral resolution. In addition, lipid identifications were obtained in consecutive slides on an Orbitrap Elite™ Hybrid Ion Trap-Orbitrap Mass Spectrometer in data dependent acquisition mode. Data were analyzed using MATLAB and Lipostar software.

Results: Principal component and discriminant analysis showed that different lipid species were responsible for the discrimination between OA and CD patients. Phosphocholines and sphingomyelins were more abundant in the OA group, whereas phosphoethanolamines and phosphoserines were more common in the CD group. Furthermore, molecular intra-tissue heterogeneity was found for adipose-, connective- and synovial tissue.

Conclusions: Our results suggest that lipid analysis in HFP using MALDI-MSI could be a useful source for biomarker detection in predicting cartilage regeneration and OA development.

Abstract **#1172**

CADHERIN-11 AND CADHERIN-2 REGULATE MESENCHYMAL STEM CELLS DIFFERENTIATION DIFFERENTLY IN MONOLAYER CULTURE COMPARED TO AGGREGATE CULTURE

Fiona Passanha[P][1]

[1] *Maastricht University*

Corresponding author's email: f.passanha@maastrichtuniversity.nl

The acquisition of specific cell fate is one of the core aims of tissue engineering and regenerative medicine. The influence of the cell–material interface on cell fate has been an area of significant research, but comparatively little is currently known about cell–cell interaction. Furthermore, while there is significant evidence that cells cultured as aggregates positively influence fate decisions, the mechanisms underlying this are not known. Human mesenchymal stem cells (hMSCs) from bone marrow have the ability to differentiate into three lineages in vitro and are an example of a cell type that has been shown to differentiate more effectively as aggregates. Here we study aggregates of hMSCs in vitro, in which we study cadherin expression, an important class of proteins involved in cell adhesion and signaling during proliferation and differentiation into different lineages. We make a comparison to cells cultured as a monolayer. We observe that proliferating hMSCs cultured as a monolayer expressed lower levels of cadherin-2 and increased cadherin-11 expression at cell–cell contact sites over time, which was not evident in the aggregate cultures. Furthermore, the importance of the cadherins in regulating the differentiation of hMSCs is evident by their knockdown. Knocking down the cadherins impeded hMSC differentiation towards the adipogenic lineage, but cells continued to differentiate to the osteogenic lineage.

Abstract **#1173**

CADHERIN-11 REGULATES EXTRACELLULAR MATRIX PRODUCTION IN MESENCHYMAL STEM CELL

Fiona Passanha[P][1]

[1] *Maastricht University*

Corresponding author's email: f.passanha@maastrichtuniversity.nl

For regenerative medicine, directing stem cell fate is one of the key aims. Human mesenchymal stem cells (hMSCs) are versatile adult stem cells that have been proposed for several clinical applications, therefore directing their fate is of utmost importance. For most clinical applications, their differentiation towards the adipogenic lineage is an undesired effect. Cadherin-11, a cell adhesion molecule expressed by the hMSCs, is required for their commitment towards the adipogenic lineage. To investigate the mechanisms through which cadherin-11 regulates adipogenic differentiation, we studied the hMSCs and its knockdown. We observed that hMSCs lacking cadherin-11 had decreased expression of type VI collagen and fibronectin. In our study, we provide evidence of increased TGFb1 and the subsequent translocation of phosphorylated SMAD 2/3 into the nucleus by cells that lack cadherin-11, which could be attributed to the changes in extracellular matrix composition. Taken together, our study implicates cadherin-11 in regulating extracellular matrix production.

Abstract **#1176**

DEVELOPMENT, FABRICATION AND BIOLOGICAL VALIDATION OF A NEW NICHOID SCAFFOLD FOR ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

Bianca Barzaghini[P][1], Federica Rey[2], Letizia Messa[1], Francesca Fanizza[1], Roberto Osellame[3], Giulio Cerullo[3], Gian Vincenzo Zuccotti[2], Stephana Carelli[2], Manuela Teresa Raimondi[1]

[1] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy*

[2] *Pediatric Research Center "Romeo ed Enrica Invernizzi", University of Milano, Milano, Italy*

[3] *Istituto di Fotonica e Nanotecnologie (IFN)-CNR and Department of Physics, Politecnico di Milano, Milano, Italy*

Corresponding author's email: bianca.barzaghini@polimi.it

Human Adipose-Derived Stem Cells (hADSCs) represent a promising source for cell therapies because their availability is less invasive compared to the one from other organs or tissue. In the body, SCs reside in a specialized microenvironment, characterized by a unique combination of biophysical and biochemical properties. Recently, biomaterials have been used to create 3D micro-scaffolds, which mimic the biomechanical characteristics of SC niches. In this context, the micro-niche "Nichoid" has shown the ability to induce pluripotency in SCs thanks to the isotropic mechanical stimuli(1,2). The aim of this study was to investigate the proliferation and stemness properties of hADSCs expanded inside these engineered niches with a cubic-pore configuration.

Nichoids were fabricated by two-photon polymerization using a biocompatible photoresist(3). hADSCs were isolated from lipoaspiarate adipose tissue obtained by liposuction performed in voluntary subjects. Cells expanded inside the cubic Nichoid have been characterized by viability assay, Real Time PCR and immunofluorescence analysis. Furthermore, neural differentiation was also investigated.

The expansion inside the cubic 3D micro-scaffold permits the potentiation of stemness features by upregulating the expression of pluripotency genes. Furthermore, cells expanded could be easily collected in order to be transplanted.

Our findings represent a great promise for the Nichoid's application in the field of regenerative medicine, in particular in neurodegenerative diseases as Parkinson's disease and Spinal Cord Injury.

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Abstract **#1177**

DRUG RESISTANCE INDUCTION IN MICROGEL-BASED MULTIPLE MYELOMA SUSPENSION CULTURES

José Luis Gómez Ribelles[1,2], Sandra Clara-Trujillo[P][1,2], Gloria Gallego-Ferrer[1,2], Annj Zammuner[3,4]

[1] CBIT – Centre for Biomaterials and Tissue Engineering, Universitat Politècnica de València, 46022 Valencia, Spain

[2] Biomedical Research Networking Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Valencia, Spain

[3] University of Padua. Department of Industrial Engineering, Padua, Italy

[4] LIFELAB Program, Consorzio per la Ricerca Sanitaria- CORIS- Veneto Region, Via Giustianini, 2, Padua, Italy

Corresponding author's email: sanclatr@gmail.com

The development of suitable models that better recapitulate tumor microenvironment is essential to understand cancer biology and to develop effective treatments [1]. Multiple myeloma (MM) is a hematological neoplasia in which bone marrow (BM) niche depicts a key role in disease progression and drug resistance. MM cells maintenance in vitro, and the reproduction of the in vivo occurring drug resistance (DR), remains still challenging for tissue engineering approaches. The use of microspheres as 3D substrate in in vitro tissue modeling has increased prevalence in past years. In this work, a biomimetic substrate has been generated by using extracellular matrix (ECM)-coated microspheres kept in suspension (microgels) with MM cells. Polymeric microspheres have been obtained by emulsion polymerization method from acrylates and acrylic acid and functionalized with ECM molecules (fibronectin, hyaluronic acid). Suspension cultures of MM cells in the platform have been exposed to dexamethasone. The generated 3D culture system significantly increased cell proliferation in comparison with conventional 2D cultures and corroborated the development of resistance to dexamethasone under BM biomimetic conditions.These studies imply the development of an innovative 3D versatile cell culture platform, in which the impact of specific ECM formulations in MM cells proliferation and DR can be studied.Acknowledgements: PROMETEO/2016/063 project is acknowledged. This work was also supported by the Spanish Ministry of Science, Innovation and Universities trough Sandra Clara-Trujillo FPU17/05810 grant.

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Abstract **#1179**

EFFICIENT TRANSFECTION OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS VIA NIOSOMES CARRIERS.

Natalia Carballo-Pedrares[P][1], Ana Rey-Rico[2]

[1] *Centro de Investigacións Científicas Avanzadas (CICA); Universidade da Coruña (UDC).*

[2] *Centro de Investigacións CIentíficas Avanzadas (CICA); Universidade da Coruña (UDC).*

Corresponding author's email: natalia.carballo@udc.es

Nonviral systems involve the complexation of nucleic acids with positively charged gene carriers (mostly cationic polymers or lipids) to promote their cell uptake. Despite their biosafety as compared with their viral counterparts, nonviral carriers exhibit some obstacles including their fast degradation, aggregation tendency and lower transfection efficiency [1]. Among nonviral carriers, niosomes have emerged as potential candidates for gene delivery applications providing higher stability and lower toxicity compared with classical liposomes [2]. The aim of this work was to produce different niosomes formulations in order to achieve an effective transfection of human mesenchymal stem cells (hMSCs), a potential population for applications in regenerative medicine [3]. Niosomes with various helper lipids and surfactants were prepared by a reverse phase evaporation technique. Niosomes and their nioplexes with a reporter DNA lacZ plasmid (placZ) were characterized by different methodologies including agarose retardation and SYBR Green-I dye exclusion assays. Likewise, both size and charge from systems were evaluated by DLS and ELS, respectively. Results showed that niosomes formulated at 1:10 placZ /cationic lipid ratio (w/w) provided the higher transfection efficiency of hMSCs monolayers.

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Abstract **#1180**

EXTRACELLULAR MATRIX DURING PHYSIOLOGICAL AND PATHOLOGICAL CARDIAC AGEING: A PROTEOMIC STUDY

Deolinda Santinha$_P$[1], Andreia Vilaça[1,2], Annachiara De Sandre-Giovannoli[3], Xavier Nissan[4], Alessandro Ori[5], Lino Ferreira[1]

[1] *Faculty of Medicine and Center for Neuroscience and Cell Biology - University of Coimbra, Portugal*

[2] *Department of Molecular Genetics, Faculty of Science and Engineering, Faculty of Health, Medicine and Life Sciences, Maastricht, The Netherlands*

[3] *Molecular Genetics Laboratory, Department of Medical Genetics, La Timone Children's hospital, Marseille, France*

[4] *CECS, I-STEM, AFM, Institute for Stem Cell Therapy and Exploration of Monogenic diseases, Evry cedex, France*

[5] *Leibniz Institute on Ageing – Fritz Lipmann Institute (FLI), Jena, Germany*

Corresponding author's email: deosantinha@gmail.com

Ageing is a multifactorial and tissue-specific process associated with a decline of protein, cell, and organ function [1]. Ageing in the cardiovascular system is seen by researchers as the beginning of human ageing [2]; however, our understanding is relative scarce. As cardiac extracellular matrix (ECM) provides structural support for cardiac cells and regulates many cellular homeostasis and intracellular signaling processes, the aim of this work was to identify and validate cardiac ECM proteins involved in physiological and pathological ageing (premature ageing). To accomplish this goal, we have performed proteomic analyses of cardiac tissue lysates and decellularized cardiac tissue ECM from old and young mice or human cardiac tissues. The impact of pathological ageing was investigated using FFPE cardiac tissue from double mutant mice for LMNA gene (progeroid mice) and patients diagnosed with Hutchinson-Gilford progeria syndrome. We have identified physiological and pathological ageing signatures in ECM proteome in both species. Decellularized ECM allowed us to show a higher number of ECM proteins differentially regulated in physiological ageing and identify a commonly interesting hit protein inter-species which have been studied in vitro to disclose its role in cardiovascular ageing biology. The current study is the first to show the impact of ageing (physiological and pathological ageing) in cardiac ECM proteome in human and mouse. Ongoing studies will dissect the role of the ageing-affected protein in cardiovascular ageing biology and identification of new therapeutic targets. The authors would like to acknowledge the financial support through the FCT PhD Studentship (PD/BD/106051/2015) and project entitled "An iPSC-derived vascular model of Progeria to identify mediators for smooth muscle loss" (Ref:029229), and trough the ERA Chair entitled "ERA atUC: Enhancing Research in Ageing at the University of Coimbra" (Ref:669088). We further acknowledge the funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952266.

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Abstract **#1181**

TRACHEAL TISSUE ENGINEERING: IS THERE ANY PROGRESS?

Seong Keun Kwon[P][1,2]

[1] *Seoul National University Hospital*

2 Seoul National University College of Medicine

Corresponding author's email: kwon.seongkeun@gmail.com

There are no effective solutions for the treatment of advanced structural disorders of the large airways. Although tracheal resection and end to end anastomosis is the primary treatment of choice, it is technically challenging and is not applicable to long tracheal stenosis. Allo-transplantation is associated with significant long-term mortality due to infection and immunosuppression, especially in the respiratory system. There is also a paucity of donors for transplantation.

In the past decade, tissue-engineered tracheal structures re-populated with cells or stem cells have been tried. The potential clinical advantages of autologous stem-cell-derived transplants are that patients who receive them would not need immunosuppression and that the transplants are hypothesized to be remodeled by local stroma to simulate native tissue. However, most of the studies failed clinical translation due to restenosis at the anastomotic site. Thus, there is a significant unmet need for novel methods of replacing and regenerating human tracheal tissue.

The ideal endpoints for tracheal replacement are normal airway and lung function, appropriate growth, high quality of life, and the elimination of the need for repeated surgical interventions.

Here I would like to describe the series of my studies on the tracheal regeneration focusing on reducing the restenosis while enhancing angiogenesis using various stem cells, spheroid culture, 3D printed scaffold, bioreactors, bioactive drug eluting scaffolds to modulate the fibrosis pathway, and modification of transplantation technique using pedicled flap.

Abstract **#1182**

ENGINEERING OSTEOARTHRITIC CARTILAGE MODEL THROUGH DIFFERENTIATING SENESCENT HUMAN MESENCHYMAL STEM CELLS FOR TESTING DISEASE-MODIFYING DRUGS

Hang Lin[P][1], Ning Wang[1]

[1] *University of Pittsburgh*

Corresponding author's email: hal46@pitt.edu

Significant cellular senescence has been observed in cartilage harvested from patients with osteoarthritis (OA). Preclinical studies further suggest that selectively removing senescent cells attenuates the development of post-traumatic OA. However, the mechanism underlying senescence-associated cartilage degeneration has not been fully demonstrated. In this study, we aim to develop a senescence-relevant OA cartilage model to study the pathogenesis of OA and develop disease-modifying OA drugs (DMOADs). Specifically, human bone marrow-derived mesenchymal stem cells (MSCs) were expanded in vitro up to passage 10 (P10-MSCs). Following their senescent phenotype formation, P10-MSCs were subjected to pellet culture in chondrogenic medium. Results from qRT-PCR, histology and immunostaining indicated that cartilage generated from P10-MSCs displayed both senescent and OA-like phenotypes without using other OA inducing agents, when compared to that from normal passage 4 (P4)-MSCs. Interestingly, the same genetic expression differences observed between P4-MSCs and P10-MSC-derived cartilage tissues were also observed between the preserved and damaged OA cartilage regions taken from human samples, as demonstrated by RNA Sequencing data and other analysis methods. Lastly, the utility of this senescence-initiated OA cartilage model in drug development was assessed by testing several potential DMOADs and senolytics, including naproxen, SM04690, FGF18, ABT-263, as well as a senolytic cocktail: dasatinib (D)/quercetin(Q). The results suggest that pre-existing cellular senescence can induce the generation of OA-like changes in cartilage. The P4- and P10-MSCs derived cartilage models also represent a novel platform for predicting the efficacy and toxicity of potential DMOADs on both preserved and damaged cartilage in humans.

Abstract **#1183**

REGULATORY ROLE OF THE MIR-29C/H19 AXIS ON MINERALIZATION AND EXTRACELLULAR MATRIX COMPOSITION

Sara Moura[P][1,2], Jorge Lopes[3], Susana Santos[1,2], Nuno Neves[1,3], Mário Barbosa[1,2], Maria Inês Almeida[1,2]

[1] *I3S/INEB - Instituto de Investigação e Inovação em Saúde, Universidade do Porto*

[2] *ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto*

[3] *CHUSJ - Departamento de Ortopedia, Centro Hospitalar São João*

Corresponding author's email: sara.moura@i3s.up.pt

Non-coding RNA (ncRNAs) are a large class of molecules able to promote bone regeneration and to control bone homeostasis. It includes small (e.g. microRNAs) and long [lncRNAs (e.g. lincRNA)] transcripts that do not translate into proteins but can modulate cellular differentiation, proliferation, or extracellular matrix (ECM) composition. This study aims to explore the role of miR-29c, and the related lncRNAs, in the ECM secreted by human primary mesenchymal/stromal cells (hMSCs) and osteoblasts.

To achieve this goal, hMSCs were isolated from the bone marrow of osteoporotic patients that underwent total hip arthroplasty following a fragility fracture. miR-29c overexpression in hMSCs led to a decrease in the expression of several ECM components including COL1A1, COL1A2 and COL3A1, whereas miR-29c inhibition had the opposite effect. Mineralization was increased in miR-29c-overexpressing hMSC, while osteogenic marker ALP was not changed by this miRNA under osteogenic-inducing conditions. This suggests a role of miR-29c in mineralization rather than in early stages of osteogenic differentiation. Next, ENCORI platform was used to identify lncRNAs predicted to interact with miR-29c. In vitro assays showed that increased miR-29c levels negatively impacted lncRNA H19 expression. On the other hand, miR-29c silencing promoted H19 expression. Importantly, silencing of H19 led to impaired expression of collagens.

In conclusion, expression of ECM components can be potentially programmed by engineering the miR-29c/H19 axis.

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Abstract **#1184**

DEVELOPMENT OF A NEW IN VITRO DEVICE FOR RISK ASSESSMENT OF INHALED XENOBIOTICS: LUNG/LIVER

Sabrina Madiedo-Podvrsan[P][1,2], Fengping Zhu[1], Marie Cattelin[1], Thomas Martinez[1], Louise Sebillet[1], Rachid Jellali[1], Ghislaine Lacroix[2], Muriel Vayssade[1]

[1] Université de technologie de Compiègne, CNRS, Biomechanics and Bioengineering, Centre de recherche Royallieu - CS 60319 - 60203 Compiègne Cedex, France

[2] French National Institute for Industrial Environment and Risks, INERIS, Chronic Risks Division, Verneuil-en-Halatte, France

Corresponding author's email: sabrina.madiedo-podvrsan@utc.fr

Adverse effects of inhaled pollutants on human health are largely accepted however investigation methods are challenged by European regulations and the promotion of animal welfare. In response, various alternative in vitro methods are developed. Among all, body-on-a-chip systems reveal to be interesting microtools shown to better mimic physiological conditions and enable potential specific inter-tissue interaction studies.

The purpose of this project is to connect a bronchial tissue with a metabolically functional liver biochip to assess any potential toxicological interactions between both compartments during inhalation-type exposures.

The in vitro lung/liver-on-a-chip model was validated through referenced hepatotoxic exposures of acetaminophen (APAP) by monitoring various cell parameters such as viability, bronchial tissue integrity, mucin detection on the tissue surface and hepatic metabolism after 72-hour exposures. In spite of xenobiotic exposures, Calu-3 bronchial tissues and HepG2/C3A biochips show strong viability and differentiation: E-cadherin and Claudin-1 expressions and elevated transepithelial electrical resistance (TEER) values indicate cohesive bronchial tissues, MUC5A production by bronchial tissue is seemingly maintained and hepatic and pulmonary metabolisms remain active. Presence of secondary metabolites highlight successful passage and transit of APAP through the closed circuited system.

Hepatotoxic exposures through the pulmonary tissue result in observable adverse effects in both compartments, translating an active inter-compartmental communication and a sensitivity of the device to xenobiotic exposure. Characterization of the developed lung-liver-on-a-chip is promising and opens the way to new physiologically relevant applications for toxicological research such as pesticide investigation.

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Abstract **#1185**

GCN5 CONTRIBUTES TO INTRACELLULAR LIPID ACCUMULATION IN HUMAN PRIMARY CARDIAC STROMAL CELLS FROM ACM PATIENTS

Chiara Volani[P][1], Viviana Meraviglia[2], Marzia De Bortoli[2], Benedetta Ermon[2], Theresa Matzinger[2], Elisa Cogliati[3], Adolfo Paolin[3], Giulio Pompilio[4], Johannes Rainer[2], Giuseppe Paglia[2], Peter Pramstaller[2], Elena Sommariva[4], Alessandra Rossini[2]

[1] *Institute for Biomedicine, Eurac Research, Bolzano, Italy - Affiliated Institute of the University of Lübeck, Lübeck, Germany*

[2] *Institute for Biomedicine, Eurac Research, Bolzano, Italy - Affiliated Institute of the University of Lübeck, Lübeck, Germany*

[3] *Fondazione Banca dei Tessuti di Treviso Onlus, Italy*

[4] *Vascular Biology and Regenerative Medicine Unit, Centro Cardiologico Monzino Milan, Italy*

Corresponding author's email: chiara.volani@eurac.edu

GCN5 is a histone acetyltransferase with a role in adipogenesis. Arrhythmogenic Cardiomyopathy (ACM) is a genetic disease associated with sudden cardiac death and cardiac fibro-fatty replacement. Human primary cardiac stromal cells (CStCs) are known to contribute to adipogenesis in the heart of ACM patients. This study aims to evaluate whether GCN5 plays a role in intracellular lipid accumulation of ACM CStCs. CStCs are obtained from right ventricle biopsies of ACM patients and from samples of healthy cadaveric donors (CTR). Western Blot analysis reveals higher GCN5 levels in ACM CStCs compared to CTR CStCs in growth medium (ACM=1.017±0.08003, CTR=0.6187±0.09208; p<0.0044). After the exposure to Adipogenic Medium (AM) for 7 days in presence of 200 µmol/L MB-3, a specific GCN5 inhibitor, ACM CStCs show a reduction of lipid accumulation (ACM AM=1236±119.6, ACM AM+MB-3=234.3±42.19; p=5.5x10-17). Lipid accumulation is also significantly decreased in ACM CStCs after GCN5 silencing (Untrasduced=777.4±154.6, Scramble=678.4±107.4, shRNA GCN5=95.34±18.81; Untransduced vs shRNA GCN5 p=4.6x10-10; Scramble vs shRNA GCN5 p=2.04x10-23). To investigate the mechanisms involved, a transcriptomic analysis has been performed: in presence of MB-3, pathways related to metabolic processes are downregulated, while pathways related to redox balance are upregulated. In agreement, MB-3 administration is also able to reduce the mitochondrial Reactive Oxygen Species (ROS) concentration (ACM AM=4120±309.2, ACM AM+MB-3=2571±207.8; p=3.0x10-8), while the treatment of ACM CStCs with the mitochondrial ROS scavenger, MitoTempo (500 nM), significantly reduces lipid accumulation (ACM AM=1764±149.9, ACM AM+MitoTempo=1132±131.3; p=4.4x10-6). Our findings suggest that GCN5 inhibition might reduce fat accumulation of ACM CStCs, possibly by modulating redox processes.

Abstract **#1186**

MODIFICATION OF DECELLULARISATION METHODS TO ASSESS THE EFFECTS OF SWELLING ON THE MECHANICAL PROPERTIES OF PORCINE TENDON

Jacqueline Solis-Cordova$_P$[1,2], Jennifer Helen Edwards[1,2], Hazel Fermor[2], Claire Brockett[1], Anthony Herbert[1]
[1] *Institute of Medical and Biological Engineering, School of Mechanical Engineering, University of Leeds, UK*
[2] *Institute of Medical and Biological Engineering, School of Biomedical Sciences, University of Leeds, UK*
Corresponding author's email: bsbjsc@leeds.ac.uk

Anterior cruciate ligament (ACL) rupture accounts for 40% of knee injuries [1], requiring 400,000 reconstructions annually worldwide [2] Decellularised porcine superflexor tendon (pSFT) provides an off-the-shelf, cost-efficient option for ACL reconstruction (ACLR). During decellularisation, phosphate buffered saline (PBS) is used for washing out cytotoxic solutes and reagents, whilst maintaining tissue hydration. It has been shown to increase water content in tendon, swelling the tissue [3]. This has proven to reduce the mechanical properties of tendon [4, 5]

In this study, end stage PBS washes in the standard protocol were substituted with 0.9% physiological saline to determine if tissue swelling could be reduced without negatively affecting its mechanical properties, while achieving the same degree of cellular removal. pSFTs decellularised using the modified protocol were compared to pSFTs decellularised using the standard protocol and native tendons.

Geometrical measurements and weight exposed no significant differences in tissue swelling between decellularised groups, yet less percent increase in width and thickness for the modified group. Histological evaluation showed efficient decellularisation in both groups, with no observational differences in histoarchitecture. Stress relaxation testing resulted in reduced relaxation moduli for both decellularised groups. The mechanical properties obtained from strength testing were not significantly different compared to native pSFTs or between them. However, the toe region modulus of the modified group was significantly different to both native pSFTs and standard group. Decellularised pSFTs using the standard or modified process have suitable mechanical properties to act as a viable graft for ACLR.

Keywords
anterior cruciate ligament; tendon; decellularisation

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Abstract **#1187**

A METHOD TO GENERATE PERFUSABLE PHYSIOLOGIC-LIKE VASCULAR CHANNELS WITHIN ORGANS-ON-CHIP

Erika Ferrari[P][1], Roberta Visone[1], Paola Occhetta[1], Marco Rasponi[1]
[1] *Department of Electronics, Information and Bioengineering. Politecnico di Milano. Via Golgi 39, 20133, Italy*
Corresponding author's email: erika1.ferrari@polimi.it

The recapitulation of 3-dimensional (3D) architecture of native organs, including their vasculature, is paramount for the generation of functional in vitro models. Advanced Organs-on-Chip (OoC) platforms have been recently developed, able to provide physical stimulations to enhance tissue-specific functionality. However, these models present either separating membranes[1] or scaffolds[2] between tissues and endothelial cells, introducing physical barriers not present in the native milieu. Moreover, they fail to recapitulate the intrinsic circularity of vascular channels, which is known to promote barrier functionality and cytoskeletal alignment of endothelial cells[3]. We here developed a method to generate on-chip a perfusable circular endothelial channel embedded in a 3D construct without any physical separation. The device comprises a 720µm central culture chamber, flanked by two lateral channels for medium replenishment, separated by rows of hanging posts. We implemented the method in a novel Liver-on-Chip platform: hepatocytes (HepaRG) were embedded in a fibrin gel, generating a 3D construct surrounding an inner vascular channel, lined by endothelial cells (HUVECs). Liver functionality was assessed through albumin, enzymatic assays and immunofluorescence staining of peculiar hepatic biomarkers. Electrodes were added to assess the generated hepatic-endothelial barrier through transendothelial electrical resistance (TEER) measurements. The 3D vascularized liver model, suitable for drug screening applications, is also amenable to study the behavior of the immune component flowing in the vasculature in phato-physiological conditions. The proposed technology is compatible with the uBeat® technology[4], providing microtissues with a controlled mechanical stimulation thus resulting suitable with other applications (e.g. heart, gut) in the OoC field.

Keywords
vasculature; liver-on-chip; perfusion

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Abstract **#1188**

SUPRAMOLECULAR COLLOIDAL HYDROGELS FOR DESIGN OF FUNCTIONAL ADAPTIVE BIOMATERIALS

Andrij Pich$_P$[1,2,3]

[1] *RWTH Aachen University*

[2] *DWI Leibniz Institute for Interactive Materials e.V.*

[3] *Aachen Maastricht Institute for Biobased Materials, Maastricht University*

Corresponding author's email: pich@dwi.rwth-aachen.de

Aqueous microgels exhibit unique properties like high chemical functionality, reversible deformability, surface activity, and stimuli-responsiveness. Functional microgels can be used as building blocks for the preparation of well-ordered nanostructured biomaterials of different dimensions and complexity.

This contribution will focus on stimuli-responsive microgels exhibiting non-covalent dynamic crosslinks based on host-guest complexes, ionic bonds or hydrogen bonds. The development of new synthesis methods that allow controlled integration of supramolecular functionalities into microgels opens new ways to generate functional polymer materials and systems with unique functions like stimuli-responsiveness, re-shaping, and triggered disassembly.

Monodisperse colloidally stable microgels with a high amount (> 30 mol-%) of zwitterionic groups were synthesized using W/O miniemulsion approach. High contents of zwitterionic groups in microgels led to the formation of dynamic reversible ionic crosslinks along with permanent covalent crosslinks generated by bisacrylamide. Obtained microgels exhibit temperature-triggered swelling/deswelling behavior and co-existance of UCST- and LCST-type transitions in aqueous solutions.

A new synthesis method to obtain aqueous supramolecular temperature-responsive microgels using polyphenols as multifunctional physical cross-linker was developed recently. The precipitation polymerization of N-vinylcaprolactam in the presence of tannic acid leads to the formation of well-defined stimuli-responsive microgels cross-linked by hydrogen bonds formed between the tannic acid and carbonyl groups of the polymer chains. We demonstrate that obtained microgels exhibit reversible temperature-triggered swelling/deswelling and undergo pH-triggered degradation and re-shaping in aqueous solutions.

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Abstract **#1189**

DEVELOPMENT OF BIOMIMETIC PROTEOLIPOSOMES AS SYNTHETIC OSTEOGENIC EXTRACELLULAR VESICLES FOR BONE REGENERATION

Mathieu Brunet$_P$[1], Kenny Man[1], María Fernández-Rhodes[2], Owen Davies[2], Marie-Christine Jones[3], Sophie Cox[1]

[1] *School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*

[2] *School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, LE11 3TU, UK*

[3] *School of Pharmacy, Institute of Clinical Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*

Corresponding author's email: MYB925@student.bham.ac.uk

Extracellular vesicles (EVs) derived from mineralising osteoblasts have demonstrated their potential as osteoinductive acellular therapies bypassing the limitations associated with cell-based approaches. Nevertheless, the clinical translation of EVs is still obstructed by issues associated with their purity, homogeneity and scalability. In this study, the formulation of osteogenic proteoliposomes was investigated to develop an EV-biomimetic artificial model based on the characterization of naturally-derived osteogenic extracellular vesicles. EVs were isolated from mineralising osteoblasts over a 2-week period and their size, morphology and concentration were subsequently characterised along with the validation of EVs tetraspanin markers. The EVs pro-osteogenic capacity on promoting human mesenchymal stem cells (hMSCs) osteogenic differentiation were assessed via quantifying alkaline phosphatase activity and calcium deposition. Lipidomics and proteomics analysis were conducted to determine EV composition which guided the formulation of biomimetic proteoliposomes using the thin-film hydration method followed by extrusion. EVs proteomics analysis highlighted that several proteins such as annexins were enriched in EVs derived from mineralising osteoblasts. The proteoliposomes did not significantly affect hMSCs viability when compared to the use of naturally derived EVs. Importantly, the biomimetic synthetic vesicle membrane harboring annexin V and tissue non-specific alkaline phosphatase (TNAP) were found to be functional with the validation of the mediation of Ca2+-influx by annexin V into the nanoparticles and TNAP enzymatic activity. Taken together, these findings demonstrate the potential of proteoliposomes as a suitable candidate for the clinical translation of synthetic extracellular vesicles-based therapy for bone regeneration.

Abstract **#1190**

THE USE OF PHYSIOLOGICAL OXYGEN CONCENTRATION IN CULTURE OF ADIPOSE-DERIVED STEM CELLS MAY POSITIVELY INFLUENCE THE CHARACTERISTICS OF THE CELLS RELEVANT TO THEIR CLINICAL USE

Cezary Piwowarczyk[P][1,2], Ilona Szabłowska-Gadomska[1,2], Anna Cieciuch[1,2], Małgorzata Lewandowska-Szumieł[3,1,2]

[1] *Laboratory for Cell Research and Application, Medical University of Warsaw, Warsaw 02-097, Poland*

[2] *Centre for Preclinical Research and Technology, Warsaw 02-097, Poland*

[3] *Department of Histology and Embryology, Centre for Biostructure Research, Medical University of Warsaw, 02-004 Warsaw, Poland*

Corresponding author's email: cezary.piwowarczyk@wum.edu.pl

Adipose-derived stem cells (ADSC) are considered as the active substance of medicinal products dedicated to numerous diseases. However, despite promising outcomes from clinical trials, the effectiveness of ADSC-based therapies remains limited. According to the current state-of-the-art, the ADSC-based products are prepared under conditions of atmospheric oxygen concentration – dramatically higher when compared to conditions present in their niche. High oxygen concentration in cell culture is related to reactive oxygen species emergence increasing cell death occurrence.

The main objective of this study was to determine the effect of physiological oxygen concentration on ADSC in cell culture, as compared to ambient conditions (5%O2 vs. 20%O2, v/v). Serum-free, GMP-grade medium was used to provide resemblance to manufacturing of medicinal product. The following aspects were examined in cells cultured under both conditions in 3 time points up to day 21: proliferation (PrestoBlue Assay), selected gene expression (qPCR), DNA-damage (Immunocytochemistry), senescence markers (Flow Cytometry) and ROS presence (Immunocytochemistry).

Our findings demonstrate that oxygen concentration closer to physiological provides more suitable environment for stem cells, resulting in higher proliferation ratio, significantly slower acquisition of senescence (measured by β-galactosidase activity), 2-fold lower incidence of double stranded DNA breaks on late passages (measured by phosphorylation of histone H2A.X variant) along with higher expression of genes encoding proteins crucial in wound healing – VEGF and Collagen I. Therefore, we speculate that tailoring oxygen concentration to specific stem cells needs might ensure better outcomes of clinical applications by improving cells condition and survival ratio.

Work financed by NCBR (project STRATEGMED2/267976/13/NCBR/2015).

Abstract **#1191**

ELASTIC MATRIX REPERATIVE EFFECTS OF BONE MARROW MESENCHYMAL STEM CELLS AND DERIVATIVES IN AN IN-VIVO MODEL OF AORTIC ANEURYSMS

Shataakshi Dahal$_P$[1], Simran Dayal[2]

[1] *Lehigh University*

[2] *Lehigh Unievrsity*

Corresponding author's email: anr320@lehigh.edu

Restoring homeostasis of elastic matrix in the aorta wall through regeneration and repair is critical to reversing abdominal aortic aneurysms (AAAs), rupture-prone localized aortal expansions. This is challenged by poor tropoelastin mRNA synthesis by adult and diseased vascular smooth muscle cells (SMCs). We showed that bone marrow mesenchymal stem cells (BM-MSCs) and SMCs of specific phenotypes derived therefrom (cBM-SMCs) exhibit significant elastogenic and anti-proteolytic properties in 2-D and 3-D culture systems to provide significant paracrine pro-elastogenic and anti-proteolytic stimuli to aneurysmal SMCs. While these findings encourage prospects for use of these cells for therapeutic in situ elastic matrix repair in AAAs, there are uncertainties in their biodistribution and safety, AAA homing potential and mechanisms, and long term regenerative and anti-proteolytic/inflammatory effects in the AAA wall. We investigated the effects of single and multiple dosing of the two cell types in an elastase injury rat AAA model. From assessments based on MRI based AAA sizing, histology, cytokine arrays, ELISA, and western blot analyses, we determined that cBM-SMCs exhibit the injury homing properties of BM-MSCs, can home into the AAA wall and show better retention within. Our results indicated the potential of the cells to improve elastic matrix structure, attenuate inflammatory cytokines implicated in elastic matrix breakdown and to regress aneurysm growth. These outcomes were enhanced with repeat cell dosing. The results indicate promise of a cBM-SMC-based cell therapy for AAAs though the long-term dosing regimen must be optimized and cell delivery to the AAA wall improved for better therapeutic outcomes.

Abstract **#1193**

STEM CELL INSPIRED EXTRACELLULAR VESICLES FOR REGENERATIVE REPAIR OF VASCULAR ELASTIC MATRIX

Anand Ramamurthi[P][1], Sajeesh Thampi[2]

[1] *Lehigh University*

[2] *Lehigh Unievrsity*

Corresponding author's email: anr320@lehigh.edu

Regenerative repair of the elastic matrix is a challenge to reversing pathophysiology of proteolytic disorders including Abdominal Aortic Aneurysms (AAAs). Extracellular Vesicles (EVs) are cellular components that can facilitate intracellular communications and signaling. We have demonstrated the pro-elastogenic and anti-proteolytic benefits of adult bone marrow mesenchymal stem cells (BM-MSCs), which support a regenerative cell therapy for AAAs. We thus investigated the role of EVs in elastic matrix regenerative repair effects of human BM-MSCs. EVs isolated from BM-MSCs were characterized for their bio-physical properties, and their regenerative and anti-proteolytic effects were evaluated on cytokine-injured rat aneurysmal SMCs (EaRASMCs).

EVs sourced from MSCs displayed average particle sizes of 100 nm and TEM showed negatively stained EVs to exhibit a characteristic bilayer membrane structure and a typical size range. Western blot and exosome antibody array analysis established the presence of EV surface markers TSG101 and tetraspanins (CD63, CD9), along with the presence of mesenchymal specific cell marker, Vimentin. EVs treated EaRASMC cultures exhibited anti-proteolytic effect, by inhibiting expression of key enzymes responsible for AAA progression. EV treatment was also beneficial in upregulating the expression of Lysl oxidase(LOX), a key enzyme responsible for crosslinking of elastin precursor molecules into mature fibers, and also in increasing the deposition of total cellular elastin.

Our studies showed that EVs derived from BM-MSC source provide significant anti-proteolytic and pro-elastogenic stimuli to aneurysmal SMCs, and have potential for application as a cell-free approach to aortic aneurysm wall repair.

Abstract **#1194**

AN ADVANCED NERVE GUIDANCE CONDUIT FOR REPAIRING LARGE PERIPHERAL NERVE DEFECTS

Zuzana Kocip[1,2,3], Alan J Hibbitts[1,2,3], Simone L Kneafsey[1,2,3], Leyla Zilic[1,2,3], Gang Chen[4], Brenton Cavanagh[5], Conor T Buckley[1,2,3], Simon J Archibald[6], Fergal J O'Brien[1,2,3]

[1] Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

[2] Trinity Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland

[3] Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland

[4] Department of Physiology and Medical Physics, Centre for the Study of Neurological Disorders, Microsurgical Research and Training Facility, Royal College of Surgeons in Ireland, Dublin, Ireland

[5] Cellular and Molecular Imaging, Royal College of Surgeons in Ireland, Dublin, Ireland

[6] Integra Lifesciences, Plainsboro, New Jersey, USA

Corresponding author's email: zuzanakoci@rcsi.com

Despite its limitations, an autograft is the gold standard for the treatment of large peripheral nerve injuries (PNIs). We aimed to prepare an off-the-shelf advanced nerve guidance conduit (NGC) with capacity to regenerate PNIs as effectively, but overcoming the autograft's limitations.

Advanced NGCs were composed of an outer collagen type I (Coll) tubular shell and Coll and chondroitin-6-sulphate (CS) internal matrix that was enriched by adding extracellular matrix molecules (Coll/CS/ECM). NGCs with Coll/CS and Coll/CS/ECM were tested in vitro for their immunomodulatory and neurotrophic potential and compared with autografts in vivo in a rat critical-sized nerve defect model.

In vitro analysis showed that in comparison to Coll/CS conduits, Coll/CS/ECM significantly decreased secretion of inflammatory markers and significantly increased production of nerve growth factor, vascular endothelial growth factor and interleukin-6.

In vivo analysis showed that the response of the two NGCs to electrical stimulation was similar to the autograft group and no differences were seen in electrophysiological recordings either. Consistently, no significant differences in muscle weight loss were observed between either NGC and autograft group. Importantly, total neurofilament positive area and the number of myelinated axons within both NGCs was similar to autografts. In agreement with in vitro results, Coll/CS/ECM significantly increased vascularisation when compared to Coll/CS and autograft.

Collectively, our results demonstrated that enrichment of a NGC with ECM derived molecules resulted in a biomaterial capable of modulating immune response and increasing secretion of pro-repair molecules that ultimately resulted in bridging large PNIs to a level equivalent to an autograft.

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Abstract **#1196**

TOWARDS A PHYSIOLOGICAL THREE-DIMENSIONAL ADIPOSE TISSUE MODEL

Franziska Albrecht$_P$[1], Annika Schmid[2], Annemarie Klatt[1], Simon Heine[1], Ann-Cathrin Volz[3], Petra Kluger[1]

[1] *Reutlingen University*

[2] *University of Hohenheim*

[3] *BMG Labtech*

Corresponding author's email: franziska.albrecht@reutlingen-university.de

Adipose tissue is distributed throughout most parts of the human body, fulfilling a variety of important functions such as energy storage, hormone secretion, protection of inner organs and heat isolation. It is a lose-bound connective tissue with a heterogenous cell population next to the fat-filled adipocytes. It is known for all tissues, that extra cellular matrix (ECM) shows great influence on cell recruitment, development and maturation. Therefore, ECM is a promising candidate to create an in vivo-like surrounding for cells and gaining meaningful results with test models in vitro.

In our approach we compared different 3D models to achieve in vivo-like cell behavior regarding the phenotype in combination with characteristic adipocyte functions. Models of interest were spheroids in suspension culture and a 3D cell suspension hydrogel with a prior developed matrix. Latest, comprising the polysaccharide gellan gum blended with decellularized adipose tissue to mimic native ECM. Hydrogel set up took place via bioprinting a porous grid structure and spheroid creation by liquid-overlay-technique. In all approaches adipose derived stem cells (ASCs) were used and differentiated to adipocytes for 14 days.

The results showed a successful lipid accumulation, expression of perilipin A and peroxisome proliferator-activated receptor-γ as well as a secretion of the characteristic adipokines adiponectin and leptin for both models. For each model a superior behavior could be observed: due to the hydrogel-matrix, a spontaneous differentiation of ASCs and within spheroid culture, a higher differentiation capacity.

Further, hydrogel-matrix optimization and bioprinting of spheroids, with and without additional hydrogel-matrix, is striven.

Keywords

Adipose Tissue Engineering; Physiological 3D Model; Bioprinting

Abstract **#1197**

AN INJECTABLE VISCOELASTIC PVA-BASED HYDROGEL FOR TREATMENT OF INTERVERTEBRAL DISK DEGENERATION

Xiao Lin$_P$[1], Kang Wu[1], Lei Yang[1,2]

[1] *Orthopedic Institute, Department of Orthopedics, The First Affiliated Hospital, Soochow University*

[2] *Center for Health Science and Engineering (CHSE), School of Materials Science and Engineering, Hebei University of Technology*

Corresponding author's email: xlin@suda.edu.cn

Lower back pain predominantly that related with intervertebral disk degeneration (IDD) has significant impact on nearly 80% humans. The primary cause of IDD is the increasing loading of annulus fibrosus arising from the gradual loss of mechanical properties of degenerated nucleus pulposus (NP). Injectable hydrogel as a synthetic hydrated matrix exhibits significant application potential in the regeneration of NP due to its characteristic viscoelasticity, controllable mechanical properties and availability in minimally invasive operation. Although numerous viscoelastic materials has been developed to study the role of matrix stiffness on the differentiation of NPC phenotype, rare studies conducted in isolation of biological activity only focus on effects of matrix stiffness on nucleus pulposus cells (NPC) and nucleus pulposus tissue (NPT). In present investigation, an injectable viscoelastic PVA-based hydrogel has been exploited to study the influence of matrix stiffness on NPC and degenerated NPT. By variation of the PVA content, thermoplastic hydrogel which not only bear superior injectable performance but also different stiffness is achieved. By the approach of encapsulation of NPC with PVA-based hydrogel, the proliferative and differential capacity of NPC in the hydrogel matrix was systematically studied. Furthermore, it can be established that the low stiffness matrix can play a positive role in the regeneration of NPT compared with high stiffness matrix through minimally invasive injection therapy on the degenerated NP.

Abstract **#1198**

MIMICKING THE INTESTINAL 3D TOPOGRAPHY WITH PHOTOCROSSLINKED HYDROGEL SCAFFOLDS

María García-Díaz$_P$[1], Aina Abad[1], Núria Torras[1], Elena Martínez[1,2,3]

[1] *Biomimetic systems for cell engineering, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain*

[2] *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain*

[3] *Department of Electronics and Biomedical Engineering, University of Barcelona (UB), Barcelona, Spain*

Corresponding author's email: mgarcia@ibecbarcelona.eu

The intestinal epithelium is characterized by a complex microscale topography formed by finger-like protrusions called villi and invaginations called crypts. This organized three-dimensional (3D) architecture together with the soft mechanical properties of the intestinal tissue are essential for cell behavior and tissue function. However, the experimental in vitro modeling of the delicate intestinal architecture is limited due to the difficulty in microfabricating soft materials with complex 3D geometries, high aspect ratio and curvature using efficient and simple methods. In this work, we fabricated soft hydrogel scaffolds that accurately mimic the villus and crypt morphologies of the intestinal epithelium using a simple and moldless approach (1).

We used mixtures of polyethylene glycol diacrylate (PEGDA) and acrylic acid (AA), or PEGDA and gelatin methacryloyl (GelMA) to fabricate 3D hydrogels by a single-step photolithography process. Different photomasks were used for the fabrication of the micropillars mimicking the intestinal villi and the crypt-like invaginations. The dimensions of the villi and crypts were easily fine-tuned just by changing the energy dose. The scaffolds were directly microfabricated onto permeable membranes, enabling their assembly into cell culture inserts. Intestinal epithelial cells lines or organoid-derived primary cells covered the biomimetic scaffolds and form polarized monolayers with proper barrier properties. The use of PEGDA-GelMA blends also allowed the fabrication of cell-laden scaffolds that mimic the intestinal stromal compartment.

Through these results, we demonstrated that this microfabrication technology is a promising tool to faithfully replicate the intestinal mucosa topography with improved performance, while keeping compatibility with standard cell culture techniques.

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Abstract **#1199**

COLD PLASMA AND STEM CELLS JOINED AS A NEW TOOL IN WOUND HEALING: A PILOT STUDY ON LARGE ANIMALS

Luca Melotti[P][1], Tiziana Martinello[2], Luigi Cordaro[3], Matteo Zuin[3], Anna Perazzi[4], Emilio Martines[3], Ilaria Iacopetti[4], Marco Patruno[1]

[1] *Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (PD), Italy*

[2] *Department of Veterinary Medicine, University of Bari, Valenzano (BA), Italy*

[3] *Consorzio RFX, Padova, Italy*

[4] *Department of Animal Medicine, Production and Health, University of Padova, Legnaro (PD), Italy*

Corresponding author's email: luca.melotti.4@phd.unipd.it

Skin wound healing is a complex and dynamic process that aims to restore skin integrity. Mesenchymal stem cells (MSCs) and cold plasma (APCP) are two innovative and safe therapies that have been demonstrated to accelerate and improve the healing of wounded skin [1, 2]. Herein, we describe the application of allogeneic MSCs combined with APCP in a sheep experimental model.

Two lesions were surgically induced on the back of three sheep: one was treated with MSCs and APCP, and one with PBS (placebo). APCP was applied daily. Clinical observations with histopathological and molecular analysis were used to assess the healing process at 7, 14, 21 and 42 days.

Clinically, both groups presented similar trends in wound contraction and re-epithelialization. Treated wounds showed a higher proliferation (Ki67+) and a quicker maturation of the granulation tissue than the placebo at 14 days, probably supported by the increased gene expression of VEGF. The gene expression of collagen type I and III further confirmed these findings. At 42 days, treated wounds showed a proper distribution of skin appendages resembling unwounded skin. No signs of flogosis were detected. On the contrary, in the placebo, a less amount of skin adnexa with a hyperplastic epithelium and dermal fibrosis were observed.

The combined application of MSCs and APCP led to an appropriate development of the granulation tissue and skin adnexa, thus leading to a better quality (i.e. restitutio ad integrum) of the repaired skin respect the placebo.

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Abstract **#1200**

CELL MORPHOLOGICAL RESPONSE TO 3D TOPOGRAPHY AND CURVATURE IN ENGINEERED INTESTINAL IN VITRO MODELS

María García-Díaz$_P$[1], Gizem Altay[1], Sébastian Tosi[2], Elena Martínez[1,3,4]

[1] *Biomimetic systems for cell engineering, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain*

[2] *Advanced Digital Microscopy Core Facility, Institute for Research in Biomedicine, Barcelona, Spain*

[3] *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain*

[4] *Department of Electronics and Biomedical Engineering, University of Barcelona (UB), Barcelona, Spain*

Corresponding author's email: mgarcia@ibecbarcelona.eu

Many epithelial tissues have complex three-dimensional (3D) topographies that are inherently curved. Recent advances in microfabrication techniques have allowed the development of sophisticated in vitro models that replicate this complex architecture of the native tissues. In particular, engineered intestinal tissues often use hydrogels to mimic villi structures (1). These finger-like protrusions of a few hundred microns in height have a well-defined topography and curvature. However, the characterization of these large tissue engineered constructs at single-cell resolution is technically challenging. Confocal microscopy imaging is limited by the sample thickness, whereas routine histological procedures are not suitable for high water content samples such as hydrogels. We developed a novel embedding method that allows for the histological processing of these delicate hydrogel structures (2). Using low molecular weight poly(ethylene glycol) diacrylate (PEGDA) as embedding media, we obtained a block that could be further sectioned with vibrating microtome or cryotome faithfully preserving the villi-like structures of the scaffold. We then examined the cell morphological response to the villus-like microstructures by high-resolution imaging of the cross-sections. We analyzed, in a spatially resolved manner, the cellular and nuclear morphology along the villi. We observed that cell morphological response was highly influenced by the microstructures showing significant differences in cell height and shape at different regions of the villi that presented distinguished curvatures. These findings, which are in good agreement with the data reported for in vivo experiments on the intestinal native tissue, highlight the impact of the micron-scale topography and curvature on epithelial cell behavior.

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Abstract **#1201**

AMINE MODIFIED POLYCAPROLACTONE SCAFFOLDS FOR PERIPHERAL NERVE REPAIR

Caroline Sarah Taylor$_P$[1], Rui Chen[2], David Gregory[3], Judith Curran[4], John W Haycock[5]

[1] *The University Of Sheffield*

[2] *The University of Liverpool*

[3] *University of Sheffield*

[4] *The University of Liverpool*

[5] *The University of Sheffield*

Corresponding author's email: c.s.taylor@sheffield.ac.uk

Silanization is a low cost reproducible technique to modify a material surface. We have previously reported that the addition of 11-Aminoundecyltriethoxysilane to glass substrates significantly increased neurite outgrowth length, from primary neurons, and supported primary Schwann cell adhesion compared to plain glass substrates and those coated with 3-aminopropyl triethoxysilane. We have also shown that by changing the silane chain length, the deposition of amine groups can be controlled, affect cell responses at the submicron scale. Our previous work showed the potential of 11-Aminoundecyltriethoxysilane as a coating of biomaterials in peripheral nerve repair applications. We therefore, modified Polycaprolactone (PCL) films, and electrospun fibres, with 11-Aminoundecyltriethoxysilane. Scaffolds were characterised by water contact angle, SEM, AFM and XPS. NG108-15 neuronal cells, rat primary Schwann cells and dissociated chick dorsal root ganglia were cultured on scaffolds for 7 days. Cell viability was confirmed using live/dead assay and cells labelled with β III tubulin and for S100β to confirm phenotype and perform quantitative assays.

Scaffold characterisation confirmed the addition of 11-Aminoundecyltriethoxysilane to PCL. The addition of 11-Aminoundecyltriethoxysilane to PCL scaffolds increased cellular activity and increased number of live cells could be visualised on modified PCL scaffolds. The highest average neurite lengths were found on primary neurons, and NG108-18 neuronal cells, cultured on 11-Aminoundecyltriethoxysilane modified PCL scaffolds.

PCL scaffolds can be surface modified, using 11-Aminoundecyltriethoxysilane, to improve neuronal cell responses in vitro. Future work will involve applying this treatment to PCL scaffolds to use with synthetic nerve guide conduits for investigations in vivo.

Keywords

nerve ; synthetic polymer; surface modification

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Abstract **#1202**

MODULATING MECHANICAL AND BIOLOGICAL PARAMETERS TO INVESTIGATE PHENOTYPIC CHANGES IN HUMAN LIVER SINUSOIDAL ENDOTHELIAL CELLS

Antoine Couderc[P][1], Karim El Kirat[1], Pierre Moret[1], Cécile Legallais[1], Anne Le Goff[1], Muriel Vayssade[1]

[1] *Université de technologie de Compiègne, CNRS, Biomechanics & Bioengineering, Centre de recherche Royallieu - CS 60319 - 60203 Compiègne Cedex, France*

Corresponding author's email: antoine.couderc@utc.fr

Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells at the interface between blood and hepatocytes, forming a permeable barrier thanks to many pores called fenestrae on their membrane, to the lack of basal membrane and to their high endocytic activity. LSECs are exposed to different mechanical constraints, namely a very weak stiffness from the subendothelial space of Disse and shear stress due to blood flow.

In various liver pathologies, LSECs undergo important phenotypic changes such as loss of fenestrae, development of a basal membrane and appearance of specific markers. Similarly, these changes occur in isolated LSECs within 48 hours of in vitro culture.

This project's goal is to identify the experimental parameters needed to ensure the long-term maintenance of fenestrae.

UV-crosslinked chemically modified gelatin-based hydrogels (Gelatin Methacryloyl or GelMA) were synthesized, characterized and used as cell culture support mimicking both healthy and pathologic space of Disse by tuning the mechanical properties of the hydrogel.

Sk-Hep1 (human LSEC line) cells were successfully cultured on GelMA hydrogels. The impact of various biological factors (VEGF, latrunculin, hepatocyte-conditioned culture media, BMP-9) on the cell phenotype was investigated in terms of adhesion, spreading, proliferation, viability and presence of fenestrae (assessed by scanning electron microscopy).

The co-culture of functional LSECs with hepatocytes will be a pivotal step for liver tissue engineering. It will also enable more relevant liver on chip models that could be used as drug screening platforms in order to assess human hepatotoxicity in the early stages of drug development.

Abstract **#1203**

FROM WASTE TO GOLD: INCORPORATING HUMAN LIVER ECM INTO ELECTROSPUN PCL SCAFFOLDS FOR IN-VITRO LIVER TISSUE MODELS

Thomas SR Bate[1], Sofia Ferreira-Gonzalez[1], Tak Yung Man[1], Hannah Esser[1], Rhiannon Grant[2], Gabriel Oniscu[3], Stuart J Forbes[1], Anthony Callanan[P][1]

[1] *University of Edinburgh, Edinburgh, UK.*

[2] *Maastricht University, Maastricht, Netherlands.*

[3] *Royal Infirmary of Edinburgh, Edinburgh, UK.*

Corresponding author's email: t.bate@ed.ac.uk

Cases of liver disease have been increasing for several decades, and are now responsible for 2 million deaths globally per year 1. Demand for transplants far exceeds supply which only satisfies 10% of cases on the waiting list1. New methods for the production of in-vitro liver tissue models will help unlock potential treatments that can reduce the need for transplantation. Here we investigate further the incorporation of human liver extracellular matrix (hLECM) into 3D electrospun scaffolds, elucidating a little understood factor: donor-donor variation2. Human liver samples from five different donors have been decellularised by SDS perfusion to provide ECM . The ECM has then been lyophilised and milled to a powder before incorporating into a solution of PCL. The solutions were then electrospun into scaffolds that can support HepG2 cells. The ECM and scaffold contents were analysed using FT-IR and immunohistochemical methods. HepG2 cells were cultured on the scaffolds and tests were conducted including: cell viability, DNA quantitation, immunohistochemistry staining, albumin excretion and gene expression of key functional markers. This study shows that our method successfully obtains scaffolds containing different human liver ECM samples and allows for comparison between the different donors.
ACKNOWLEDGEMENTS: This work is funded by an EPSRC doctoral training partnership studentship, UK Regenerative Medicine Platform II [RMPII] grant MR/L022974/1 and MRC grant CCBN MR/L012766/1. This work is enabled by the sacrifice of UK organ donors and their relatives, with the assistance of NHS Organ Donation and Transplant.

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Abstract **#1204**

3D PRINTING PLATFORM FOR PERSONALIZED BIOACTIVE AND BIODEGRADABLE IMPLANTS FOR MAXILLARY BONE REGENERATION

Sergi Rey-Viñolas[P][1], Soledad Pérez-Amodio[1,2,3], Miguel A. Mateos-Timoneda[2,1,3], Elisabeth Engel[1,2,3]

[1] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10–12, 08028 Barcelona, Spain*

[2] *CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain*

[3] *Materials Science and Metallurgical Engineering, Polytechnical University of Catalonia (UPC), Maristany 16, 08019 Barcelona, Spain*

Corresponding author's email: srey@ibecbarcelona.eu

Introduction:

Metallic meshes filled with different bone grafts are one of the common treatments for maxillary bone defects. Due to their mechanical properties, they can maintain the stability of the graft during the regeneration process. Nevertheless, a second surgery is needed to remove the mesh, with all the side effects that entails. Also, when autologous bone graft is used, the possibility of side effects associated to bone harvest like pain and morbidity increase. The main objective of this industrial project is to develop patient specific bioactive and biodegradable implants to substitute current techniques.

Methods:

Different bioactive calcium phosphate-based glass microparticles (MPs) were dispersed into polycaprolactone (PCL). Then, the mixtures obtained were 3D printed by fused deposition. Scaffolds were tested with human mesenchymal stem cells (hMSCs) and human gingival fibroblasts (hGFIB). Moreover, production of proteins such us alkaline phosphatase and vascular endothelial growth factor have also been quantified.

Results:

Parallel patterned scaffolds with interconnected macro porosity and homogeneous MPs dispersion were obtained. No cytotoxicity was detected by LDH assay. High biocompatibility was achieved regarding metabolic activity for both cell types. Printability of clinical cases with complex geometries have been achieved. Printed implants prototypes show a proper structure stability.

Discussion & Conclusions:

This platform holds a promising potential to develop safe bioactive and biodegradable patient specific implants, with good biocompatible properties and response to hGFib preventing future implant exposure.

Acknowledgements:

This industrial project is being developed in collaboration with AVINENT Implant System and supported by “Doctorat Industrial” grant (2017DI076) from AGAUR.

Abstract **#1205**

INTRAVITAL NONLINEAR IMAGING OF A MINIATURIZED MINI-INVASIVE WINDOW CHAMBER

Claudio Conci[1], Emanuela Jacchetti[1], Laura Sironi[2], Maddalena Collini[2], Giuseppe Chirico[2], Giulio Cerullo[3,4], Roberto Osellame[4,3], Manuela T. Raimondi[1]

[1] *Department of Chemistry, Materials and Chemical Engineering Giulio Natta, Politecnico di Milano, Milan, Italy*

[2] *Università di Milano-Bicocca, Dipartimento di Fisica, Milan, Italy*

[3] *Department of Physics, Politecnico di Milano, Milan, Italy*

[4] *Istituto di Fotonica e Nanotecnologie (IFN)-CNR, Milan, Italy*

Corresponding author's email: claudio.conci@polimi.it

In research fields as cancer and drug/biomaterial testing, intravital microscopy is fundamental to study the evolution and the dynamics of physiological phenomena at the microscale, as the subcutaneous foreign body response in terms of cell behavior and neovascularization rate. We developed a miniaturized imaging window, the Microatlas, that implanted subcute guides cell migration and neovascularization into synthetic micro-scaffolds incorporated in the device. Those scaffolds were two-photon polymerized[1] on glass coverslips (Ø12mm), with a biocompatible resist. Micrometric reference structures allowed the microscope field-of-view repositioning at different time-points during experiments. The device implanted in living chicken embryos was inspected by fluorescence microscopy. Confocal and two-photon fluorescence observations at implantation sites demonstrated tissue growth inside the scaffolds and neovascularization with a capillary density six-time greater than untreated tissue; Second harmonic generation signal showed an oriented layer of collagen-I. In conclusion, the Microatlas guided in vivo a quantifiable localized reaction inside its scaffolds, in terms of cell repopulation, collagen and capillary formation. Thus, our device can be used as a reliable imaging window for intravital quantifications.

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Abstract **#1207**

TENDON DERIVED EXTRACELLULAR MATRIX HYDROGEL FOR INJECTION AND BIOPRINTING

Ana Liz Garcia Alves$_P$[1], Fernanda de Castro Stievani[1], Manuel Gomez-Florit[2], Syeda Mahwish Bakht[2], Jõao Pedro Hübbe Pfeifer[1], Emanuel Vitor Pereira Appolonio[1], Mariana Correa Rossi[1], Gustavo dos Santos Rosa[1], Rui Domingues[2], Manuela Estima Gomes[2]

[1] *São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu*

[2] *I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal*

Corresponding author's email: ana.liz@unesp.br

The objective of this study is to preliminary evaluate the potential of decellularized extracellular matrix (dECM) from equine tendon as injectable hydrogels and bioink materials. Superficial flexor digital tendon of horses, dead from unrelated causes were aseptically harvested, chopped in slices and stored at -80oC. For decellularization, the slices were freeze-thawed prior to Triton X100 incubation, followed by lyophilization and milling (1; 2). Hydrogels were produced by first digesting the dECM powder with HCl and pepsin for 48 hours, followed by its cold neutralization with NaOH 0.2 M. Different concentration of dECM (1 and 2 wt.%) stabilized by transglutaminase (1 wt.%) crosslinking were used to produce the hydrogels (3). The microstructure was evaluated with confocal and scanning electron microscopy (SEM). The injectability and printability using 25G needle were macroscopically evaluated and hydrogels rheological behavior were characterized. Hydrogel fibrillation was confirmed by confocal and SEM, showing porous structure with well-defined nanoscale fibrils. However, while 2 wt.% hydrogels showed good integrity and permitted easy manipulation, 1 wt.% hydrogels were fragile. These characteristics were also reflected on their rheological properties, which showed higher viscosity and storage modulus for the 2% dECM, that can be tuned by transglutaminase crosslinking. Moreover, combined with 3D printing techniques in agarose-based support bath, these injectable hydrogels derived from equine tendon dECM allow to print 3D structures with good resolution. Altogether, these results suggest that equine dECM might be a promising biomaterial to be used as cell carrier in injectable formulation or bioinks.

Keywords

Tissue engineering; Bioink; Hydrogel

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Abstract **#1209**

RETINAL TISSUE BIO-ENGINEERING FROM HUMAN PLURIPOTENT STEM CELLS AS A SUBSTITUTIVE STRATEGY FOR RETINAL DEGENERATIVE DISEASE.

Elise Herardot$_P$[1], Frédéric Hamouda[2], Lise Morizur[1], Elie Frank[1], Alexandra Plancheron[1], Walter Habeler[1], Margot Jarrige[1], Hélène Polvèche[1], Alexandre Carteron[1], Dominique Decanini[2], Christelle Monville[1], Karim Ben M'Barek[1]

[1] *I-Stem, AFM, INSERM/UEVE UMR 861, Corbeil-Essonnes, 91100, France.*

[2] *Centre de Nanosciences et de Nanotechnologies (C2N), CNRS UMR 9001, Université Paris-Sud, Université Paris-Saclay, Palaiseau, 91120, France.*

Corresponding author's email: eherardot@istem.fr

In Age-related Macular Degeneration (AMD) and Retinitis Pigmentosa (RP), photoreceptors (PRs) are susceptible to degeneration due to their malfunction or because of a primary dysfunction of the retinal pigment epithelium (RPE). This cell loss is irreversible and leads ultimately to blindness. Cell therapy, which consists in the replacement of dead cells by other cells generated in vitro, is a promising therapeutic option. We recently developed a RPE therapy that could target a small part of the RP population (5%). The next step is to obtain a functional bio-engineered retina containing both RPE and PRs that could be suitable to treat the majority of RP patients and late stage AMD patients.

To generate this complex retinal tissue, we developed a protocol to produce PRs from human pluripotent stem cells (hPSCs) compatible with a clinical application. Using a combinatorial screening approach, we obtained a pure population of retinal progenitor cells co-expressing the genes PAX6 and VSX2 after 2 weeks of differentiation. This co-expression was evaluated by immunofluorescence assays. A transcriptomic analysis confirmed the retinal fate specification of differentiated hPSCs. After 7 weeks of differentiation, we also obtained immature PRs expressing Crx and Recoverin. We are pursuing this combinatorial screening strategy to obtain pure and functional PR cultures.

To assemble RPE and PR cells, we developed a micro-structured polymeric scaffold composed of regularly spaced holes where PRs could develop outer segments. Once established, this complex retinal tissue will be transplanted into blind rodent models of inherited degeneration to evaluate its therapeutic activity.

Keywords

retinitis pigmentosa; regenerative medicine

Abstract **#1210**

HYDROGEL-BASED BIOINKS FOR CELL ELECTROWRITING OF WELL-ORGANIZED LIVING STRUCTURES WITH MICROMETER-SCALE RESOLUTION

Miguel Castilho[P][1,2], Riccardo Levato[1], Paulina Nunez Bernal[1], Mylène de Ruijter[1], Christina Y. Sheng[1], Joost van Duijn[1], Susanna Piluso[1], Keita Ito[2], Jos Malda[1]

[1] *University Medical Center Utrecht*

[2] *Eindhoven University of Technology*

Corresponding author's email: M.DiasCastilho@umcutrecht.nl

Bioprinting is an emerging technique for the fabrication of biological constructs that can be used in regenerative medicine and in vitro drug testing1. However, until today, extrusion-based bioprinting processes are limited to resolutions of hundreds of micrometers, which hampers the reproduction of intrinsic functions and morphologies of living tissues2,3. This study describes an electrohydrodynamic process, named cell electrowriting (CEW), that relies on the generation of cell-laden fibers with diameters between 5- 40 µm based on an electrical field to fabricate organized three-dimensional (3D) structures. Versatility of the CEW process is demonstrated by the use of two photoresponsive hydrogel bioinks, i.e., based on gelatin and silk fibroin, which display different gelation chemistries. The rapid photo-mediated crosslinking mechanisms, electrical conductivity and viscosity of these two engineered bioinks allow the fabrication of 3D ordered fiber constructs with pores down to 100 µm with different geometries (e.g. hexagons and curved-patterns) of relevant thicknesses (up to 200 µm). Importantly, the biocompatibility of the gelatin- and silk fibroin-based bioinks enable the fabrication of cell-laden constructs, while maintaining high cell viability post-printing. Taken together, CEW opens up fascinating opportunities to manufacture microstructure scaffolds that can better resemble cellular microenvironments for regenerative medicine and organ-on-a chip models.

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Abstract **#1211**

OPTIMIZED REPROGRAMMING OF HUMAN IPSCS TO GENERATE DISTINCT NEURONAL SUBTYPES

Tony Oosterveen$_P$[1], Muhammad Kaiser Abdul Karim[1], Dumitra Bors[1], Marcos Herrera-Vaquero[1], Imbisaat Geti[1], Tuzer Kalkan[1], Farah Patell-Socha[1], Thomas Moreau[1], Mark Kotter[1]

[1] *Bit.bio, The Dorothy Hodgkin Building, Babraham Research Campus, Cambridge, CB22 3FH, UK*

Corresponding author's email: tony.oosterveen@bit.bio

Human brains differ remarkably in size and cellular composition from rodent models used in pre-clinical research. For example, human cortical neurons have larger dendritic trees, distinct electrophysical properties and express different protein isoforms. For neuronal indications, less than 10% of findings derived from animal models can be translated to the clinic. A robust source of human iPSC-derived neurons would offer an attractive in vitro model for basic research and high content drug screens, which could reduce costs, improve screen specificity, and accelerate drug development. However, conventional iPSC differentiation protocols are often complex inconsistent, and difficult to scale. To overcome these problems, we developed a proprietary gene-targeting strategy (opti-ox) that enables highly controlled expression of transcription factors to rapidly reprogram human iPSCs (hiPSCs) into pure somatic cell types. We have manufactured consistent and homogenous cultures of glutamatergic neurons (>80% VGLUT1/2) and GABAergic neurons (>95% VGAT1, GAD2), which show homogenous molecular phenotype at single cell transcriptomics resolution. Reprogramming is highly consistent and synchronised, yielding fully functional neurons in less than 14 days. Our technology opens up novel avenues for the development of in vitro models to support research and healthcare innovations.

Abstract **#1212**

CRE/LOX-ASSISTED TRACKING OF CARDIAC INTERCELLULAR COMMUNICATION

Lara Ottaviani[1], Vasco Sampaio Pinto[1], Kevin Knoops[2], Kristiaan Wouters[3], Paula A. da Costa Martins[1], Ricardo Abreu[P]

[1] *Department of Molecular Genetics, Faculty of Sciences and Engineering, Maastricht University, Maastricht, The Netherlands*

[2] *The Maastricht Multimodal Molecular Imaging Institute (M4I), Microscope CORE lab, Maastricht University, Maastricht, The Netherlands*

[3] *Dept. of Internal Medicine, MUMC, Maastricht, the Netherlands; CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University*

Corresponding author's email: lara.ottav@gmail.com

Extracellular vesicles (EVs), heterogenous lipid bilayer-enclosed structures released from a broad range of cell types, are stable carriers of small molecules. Despite promising results in the use of EVs to stimulate cardiac regeneration and protection, a better understanding of endogenous EV biogenesis and their release remains crucial when translating these therapies into the clinic. Here, we established a novel way to visualize and study EVs in the myocardium by using a Cre-loxP recombination system. Endothelial cells (ECs) were infected with an adenovirus (ADV CMV-LoxP-DsRed-LoxP-GFP) and became red (reporter+). Transducing these reporter+ cells with a second ADV (CMV-CRE), confirmed that recombination at the loxP sites induces a colour shift, from red to green. We also transduced neonatal rat cardiomyocytes (NRCMs) with an adeno-associated virus 6 (AAV6 cTNT-CFP-CRE) and verified that after 24h, >90% of the cells became CFP+. When co-culturing both cells, we observed that CFP+ NRCMs, in the upper compartment, released Cre-enriched vesicles that were taken up by endothelial reporter+ cells in the lower compartment, inducing a color switch from red to green. These data demonstrate that not only CMs can secrete Cre-containing vesicles, but also that these EVs have a functional role in the recipient (endothelial) cells. These are the first steps towards a more complex in vivo system to trace cardiac endogenous EVs in reporter mice, allowing for tracking of vesicle biogenesis, trafficking, biodistribution and timing in the heart.

Abstract **#1213**

DESIGNING THREE-DIMENSIONAL ANISOTROPIC COLLAGEN CARDIAC PATCHES USING A FREEZE-DRYING TECHNIQUE

Huijie Zhang$_P$[1], Serena Best[1], Ruth Cameron[1]

[1] *University of Cambridge*

Corresponding author's email: hz357@cam.ac.uk

Heart attacks cause irreversible damage to myocardium. There are very few treatment options available to fully restore cardiac function, except heart transplantation. However, cardiac patches have been under intense research in recent years as a potential route to repair tissue. By design, scaffolds should ideally be similar to the extracellular matrix (ECM) of myocardium. This presentation focuses on work undertaken to develop and characterize three-dimensional collagen heart patches that have comparable architectural and mechanical properties to the natural tissue.

A liquid nitrogen freeze-drying technique was used to produce collagen scaffolds with aligned porosity, offering the potential to fabricate more complicated structures for personalized heart patches and improving histological integration. X-ray microtomography suggested that by controlling the temperature profile during the freezing stage, anisotropic scaffolds with pore sizes from 65-249 micrometre could be made using this method, providing flexibilities in choosing cell types and also allowing for vascular network penetration. Chemical crosslinking was applied, and the elastic modulus was characterized by compression tests in water to simulate in vivo situation. The modulus of non-crosslinked scaffolds was within 1.4-3.8 kPa, and crosslinking was found to improve the modulus up to 6 kPa. Surprisingly, pore size had negligible effects on modulus within the range of 76-140 micrometre. Moreover, the pore alignment orientation was found to affect measured modulus, showing mechanical anisotropy, which is also observed in natural tissue.

By understanding how to manipulate scaffold structures and tune mechanical properties, we offer the possibility of replicating myocardium ECM for cardiac patch development.

Abstract **#1214**

VULNERABILITY OF PROGEROID SMOOTH MUSCLE CELLS TO ARTERIAL SHEAR STRESS IS MEDIATED BY MMP13

Patricia Pitrez[P][1,2], Luis Estronca[1,2], Luis Monteiro[1], Deolinda Santinha[1,2], Karim Harhouri[3], Claire Navarro[4], Anne-Laure Egesipe[5], Tânia Cavalho[6], João-Pedro Magalhães[1,7], Alessandro Ori[8], Andreia Bernardo[9], Annachiara De Sandre-Giovannoli[3], Xavier Nissan[5], Anna Rosell[10], Lino Ferreira[1,2]

[1] Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

[2] Faculty of Medicine, University of Coimbra, 3000-548, Coimbra, Portugal

[3] Molecular Genetics Laboratory, Department of Medical Genetics, La Timone Children's hospital, Marseille, France

[4] Aix Marseille Univ, INSERM, GMGF, Marseille, France

[5] CECS, I-STEM, AFM, Institute for Stem Cell Therapy and Exploration of Monogenic diseases, Evry cedex, France

[6] IMM, Instituto de Medicina Molecular, Universidade de Lisboa, Lisbon, Portugal

[7] Integrative Genomics of Ageing Group, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool L7 8TX, United Kingdom

[8] Leibniz Institute on Aging-Fritz Lipmann Institute, 07745 Jena, Germany

[9] Developmental Biology Laboratory, The Francis Crick Institute, London, NW1 1AT UK

[10] Neurovascular Research Laboratory. Vall d'Hebron Research institute. Passeig Vall d'Hebron 119-129, 08035, Barcelona, Spain

Corresponding author's email: pitrezpatricia@gmail.com

Hutchinson-Gilford Progeria syndrome (Progeria) is a rare premature aging disease that leads to early death. Smooth muscle cells (SMCs) are the most affected cells in Progeria patients, although the reason for such sensitivity remains poorly understood. We developed an in vitro mono-culture cell system to study the susceptibility of Progeria SMCs to arterial flow shear stress using a microfluidic device. Progeria SMCs derived from iPSCs could recapitulate the most important aspect of the disease, i.e., Progeria SMCs loss under flow shear stress. Microarray analysis comparing Progeria SMCs cultured in static conditions and under slow conditions reveals that Progeria SMCs have significant changes in extracellular matrix secretion, specifically an enzyme, MMP13. Moreover, Progeria SMC detachment is prevented by the inhibition of this enzyme. Importantly, double mutant LmnaG609G/G609GMmp13-/- mice or LmnaG609G/G609GMmp13+/+ mice treated with a MMP inhibitor showed lower SMC loss in the aortic arch than controls. Our results offer a new platform for developing treatments for HGPS individuals that may complement previous pre-clinical and clinical treatments. To the best of our knowledge, this is the first study documenting part of the mechanism underlining the sensitivity of HGPS SMCs to arterial flow shear stress.

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Abstract **#1216**

DNA-INSPIRED NANO-MATRIX FOR BIOFABRICATION IN SPACE AND ON EARTH

Yupeng Chen[P][1], Anne Yau[1], Ian Sands[1]

[1] *University of Connecticut*

Corresponding author's email: yupeng.chen@uconn.edu

Articular cartilage has limited regenerative ability, composing a minimal number of chondrocytes and components of dense extracellular matrices (ECM). The absence of biomechanical loading caused by joint immobilization on Earth or microgravity in space can damage chondrocyte functions and degrade cartilage tissue. Scientists have investigated various types of biomaterials along with stem cells and biological factors to fabricate engineered cartilage. However, it is still challenging to maintain long-term homeostasis of the engineered cartilage tissue. The key question is how to promote stem cells chondrogenic differentiation in the early stage and then inhibit their hypertrophy after differentiated.

Relying on DNA nanotechnology, our lab has developed a library of Nano-Matrices, composed of Janus-based nanotubes (JBNTs) and ECM proteins to create a biomimetic microenvironment for stem cell growth and functions. In this study, the JBNTs, mimic collagen fibers, can self-assemble with Matrilin (MATN), creating an optimal microenvironment selectively for chondrogenic differentiation and chondro-lineage cell growth. Moreover, our nanomaterials can deliver a mechano-sensitive miRNA to overcome the absence of biomechanical loading in space or on Earth. Therefore, it would enhance chondrogenesis while inhibiting hypertrophy to achieve long-term homeostasis of a cartilage tissue construct.

Furthermore, the Janus base Nano-Matrices are injectable scaffold which can be easily applied to the “difficult-to-reach” locations such as deep tissue injuries and the microchannels of tissue chips. A further goal of our research is to biofabricate an engineered cartilage tissue constructs to mimic native tissues in tissue chips for a variety of applications both in space and on Earth.

Abstract **#1217**

MOULDING THE FUTURE OF 3D PRINTING: SACRIFICIAL MOULDS FOR THE FABRICATION OF HYDROGEL CONSTRUCTS

Jan W. Robering[P][1], Supachai Reakasame[2], Jie Gao[1], Justus P. Beier[1], Aldo R. Boccaccini[2], Raymund E. Horch[3], Anja M. Boos[1]

[1] *Department of Plastic Surgery, Hand Surgery, Burn Center, University Hospital RWTH Aachen*

[2] *Department of Materials Science and Engineering, Friedrich-Alexander University Erlangen-Nürnberg*

[3] *Department of Plastic and Hand Surgery, Laboratory for Tissue Engineering and Regenerative Medicine, Friedrich-Alexander University Erlangen-Nürnberg*

Corresponding author's email: jrobering@ukaachen.de

3D printing of hydrogels is a method that is applied with increasing frequency in the field of regenerative medicine. Unfortunately, many of the hydrogels that are used traditionally for 3D cultures like collagen and fibrin are only poorly suitable for printing 3D structures. To overcome this problem, we established a blend of methyl cellulose and gelatine (Gel-MC) that can be applied as a 3D-printable sacrificial gel. By studying the rheological properties of different blends of Gel-MC we were able to optimize the printing condition for the gel and to use it, to 3D print sacrificial moulds fitting for our in vivo model of choice – the arteriovenous loop model of the rat. By loading the moulds with collagen or fibrinogen-solutions and dissolving the Gel-MC in cell culture medium, we were able to fabricate cell-laden negatives of the printed moulds. Tetrazolium-salt assays demonstrated that the Gel-MC blend is not harmful for various primary cell types. By culturing the constructs with cells in vitro, we assessed the stability and the uniformity of the fabricated fibrin and collagen constructs. Finally, we created a hydrogel implant for the arteriovenous loop model consisting of multiple functionalized parts, made from different types of hydrogel with different properties. Histological staining of the explants demonstrated tissue ingrowth as well as superior degradation properties when compared to implants made in one piece. We were able to show that printable sacrificial hydrogels for the fabrication of 3D fibrin or collagen constructs are a promising alternative to directly printed hydrogel structures.

Keywords

Collagen; Fibrinogen

Abstract **#1218**

ADHESIVE BIOCERAMICS WITH PHOSPHOSERINE: AN ALTERNATIVE, BIOINSPIRED PATHWAY TO STRONG, BIODEGRADABLE, WET-FIELD TISSUE ADHESION.

Michael Pujari-Palmer[1], Philip Procter[1], Christopher D Spicer[2], Renny Mathew[3], Gry Hulsart-Billstrom[4], Hélène Autefage[5], Gerard Insley[1], Omar Omar[6], Molly M. Stevens[5], Peter Thomsen[6], Baltzar Stevensson[3], Mattias Edén[3], Håkan Engqvist[1]

[1] *Applied Material Science, Department of Material Science and Engineering, Uppsala University, Uppsala, Sweden*

[2] *Department of Chemistry York Biomedical Research Institute University of York Heslington, UK*

[3] *Department of Materials and Environmental Chemistry, Stockholm University, Stockholm, Sweden*

[4] *Department of Surgical Sciences, Faculty of Medicine and Pharmacy, Uppsala University, Uppsala, Sweden*

[5] *Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*

[6] *Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

Corresponding author's email: michael.palmer@angstrom.uu.se

A number of bioinspired, synthetic, tissue adhesives have been created by incorporating 3,4-dihydroxy-L-phenylalanine (L-dopa), a modified amino acid that produces chemical bonding, in marine adhesives [1]. We present evidence that strong, biocompatible, wet field adhesives can be created by combining a phosphorylated amino acid that is highly enriched in marine bioadhesives, phosphoserine, with calcium phosphate cements [2]. Phosphoserine modified cements (PMCs) strongly adhere to a wide range of tissues, including bone and biomaterials (i.e. metals, polymers), and remodel in vitro, and in vivo [3, 4].

PMCs are tacky, viscous, pastes that strongly bond calcified tissues and biomaterials (2-5 MPa shear) during the setting reaction, and cure as hard, strong ceramics (50-100 MPa compression). The wet bond strength is stronger than the shear strength of human cancellous bone in many regions of the body. In fractured rodent femurs PMC stimulated robust healing, tissue remodeling (in vivo), and strong adhesion (pull-out, compared to fibrin glue, ex vivo), with failure occurring in cancellous bone and not PMC. NMR and SEM analyses suggested that the adhesive effect arose, partially, from the unique, amorphous microstructure, which included a nanoscale mineral surface layer that readily wets most surfaces. Using targeted manipulation of reactive chemical moieties (e.g. ortho/ para substitution) the adhesive strength could be finely tuned, suggesting that successive iterations of PMC with stronger calcified tissue adhesive strength can be created by via rational design of chemical reactivity.

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Abstract **#1219**

CARDIOMYOCYTE-DERIVED MICRORNA-30D REGULATES CARDIAC ANGIOGENESIS BY INHIBITING ENDOTHELIAL CELL MIGRATION

Raquel Figuinha Videira[1,2], Lara Ottaviani[1], Inês Falcão-Pires[2], Paula da Costa Martins[1,3]

[1] *1Department of Molecular Genetics, Faculty of Sciences and Engineering, Maastricht University, Maastricht, Netherlands*

[2] *2Cardiovascular Research and Development Center, Faculty of Medicine, University of Porto, Porto, Portugal*

[3] *3Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Porto, Portugal*

Corresponding author's email: r.figuinhavideira@maastrichtuniversity.nl

Background: Pathologic hypertrophy induces the release of anti-angiogenic factors from cardiomyocytes (CM) to endothelial cells (ECs), leading to impaired angiogenesis and ultimately to the development of heart failure (HF). Intercellular communication via extracellular vesicles (EVs) allows the exchange of small molecules, such as microRNAs. These non-coding RNAs control gene expression and are powerful regulators of several biological processes such as angiogenesis. The study of EV-mediated microRNA transfer that occurs during cardiac hypertrophy will help identify new therapeutic targets for HF.

Aim: Understand the role of miR-30d-3p in the angiogenic processes that occur during pathological hypertrophy towards HF.

Methods and Results: By performing a qPCR-based screening we observed that under stress conditions, CMs are able to release EVs that are enriched in miR-30d-3p. Functional assays to evaluate endothelial angiogenic capacity upon gain-of-function, using precursor molecules, or loss-of-function, using LNA inhibitors, of miR-30d-3p revealed that upregulation of miR-30d inhibits endothelial cell migration and tube formation. The potential targets and mechanism of action of miR-30d-3p were investigated by RNA-seq on HUVECs after miR-30d-3p modulation. Results suggest that miR-30d exerts its function by targeting cytoskeleton-related genes. In fact, a proliferation functional screening, using a siRNA library directed against potential targets of miR-30d, resulted in significant decrease of EC proliferative capacity.

Conclusion: Hypertrophic CMs-derived EVs carry microRNAs that induce deleterious effects on ECs and cardiac angiogenesis, miR-30d-3p being one of them. Modulation of vesicle content and/or specific microRNAs on the recipient cells might constitute a novel therapeutic strategy for the treatment of HF.

Keywords

MicroRNAs; Extracellular Vesicles; Angiogenesis

Abstract **#1220**

BIOACTIVITY AND GUIDANCE OF MUSCLE TISSUE FORMATION ON ELECTROCONDUCTIVE POLY(E-CAPROLACTONE) (PCL)-POLYANILINE (PANI) ELECTROSPUN SCAFFOLDS

Carlos A.V. Rodrigues[P][1], David Barata[2], Fábio F.F. Garrudo[1], João C. Silva[1], Katharina Hennig[2], Inês Martins[2], Joana Romão[2], Robert J Linhardt[3], Joaquim M.S. Cabral[1], Edgar Gomes[2], William Roman[2], Frederico C. Ferreira[1]

[1] *IBB – Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Portugal*

[2] *Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal*

[3] *Department of Chemistry and Chemical Biology, Biological Sciences, Biomedical Engineering and Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, USA*

Corresponding author's email: carlos.rodrigues@tecnico.ulisboa.pt

Electrically conductive poly(ε-caprolactone) (PCL)-polyaniline (PANI) blend electrospun scaffolds have been previously described for their potential use in neural tissue engineering applications (1). Further processing enabled the production of coaxial fibers (951±465 nm), which are both electroconductive (0.063±0.029 S cm-1), soft (1.3 MPa) and biodegradable. The versatility of this biomaterial platform, which can be produced as aligned fiber meshes with tunable fiber diameters, conductivities, mechanical properties and degradability rates is also interesting for growth and regeneration of muscle tissue. In this work, we have studied the ability of PCL-PANI electrospun fiber scaffolds to mimic the microenvironment of native muscle tissue in order to support adhesion and growth of myoblasts and maturation into multinucleated, contractile myofibers (2, 3). Preliminary results showed high biocompatibility of mouse primary myoblasts with the biomaterial substrate promoting cell growth over the maturation timeline. After 3 days of differentiation, the formation of aligned small myotubes was observed, some already exhibiting striations at cytoskeleton level. Further experiments based on fluorescence microscopy are currently ongoing to evaluate the structural maturity of the muscle tissue culture, setting the basis to study the effects of additional external stimuli (e.g. electrical stimulation) on the artificial muscle tissue fabrication.

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Keywords

Electrically conductive polymers; nanofibers; muscle tissue

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Abstract **#1221**

BIOPRINTING IN SPACE

Vladimir Mironov[P][1]

[1] *3D Bioprinting Solutions, Moscow, Russia*

Corresponding author's email: vladimir.mironov54@gmail.com

Magnetic levitational bioassembly of three-dimensional (3D) tissue constructs represents a rapidly emerging scaffold- and label-free approach and alternative conceptual advance in tissue engineering. The magnetic bioassembler has been designed, developed, and certified for life space research. To the best of our knowledge, 3D tissue constructs have been biofabricated for the first time in space under microgravity from tissue spheroids consisting of human chondrocytes. Bioassembly and sequential tissue spheroid fusion presented a good agreement with developed predictive mathematical models and computer simulations. Tissue constructs demonstrated good viability and advanced stages of tissue spheroid fusion process. Thus, our data strongly suggest that scaffold-free formative biofabrication using magnetic fields is a feasible alternative to traditional scaffold-based approaches, hinting a new perspective avenue of research that could significantly advance tissue engineering. Magnetic levitational bioassembly in space can also advance space life science and space regenerative medicine.

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Abstract **#1222**

NOVEL HEMOCOMPATIBLE THIOL-YNE BASED PHOTOPOLYMERS OBTAINED BY THE ADVANCED STEREOLITHOGRAPHY (SLA) PROCESSING WITH STRONGLY IMPROVED SURFACE SMOOTHNESS BY A NOVEL EXPOSITION APPROACH FOR ANTI-ALIASING

Roman Major$_P$[1], Marcin Surmiak[2], Romana Schwarz[3], Marcin Kot[4], Juergen M Lackner[5]

[1] *Institute of Metallurgy and Materials Science, Polish Academy of Sciences, Reymonta St. 25, 30-059 Cracow, Poland*

[2] *Department of Medicine, Jagiellonian University Medical College, 8 Skawinska Street, 31-066 Cracow, Poland*

[3] *Montanuniversität Leoben // Department Kunststofftechnik Lehrstuhl für Chemie der Kunststoffe Otto Glöckel-Straße 2/IV 8700 Leoben*

[4] *Faculty of Mechanical Engineering and Robotics, AGH University of Science and Technology, Mickiewicza Str. 30, Cracow, Poland*

[5] *Joanneum Research Forschungsges.m.b.H., Institute of Surface Technologies and Photonics, Functional Surfaces, Leobner Str. 94, Niklasdorf, Austria*

Corresponding author's email: r.major@imim.pl

Heart failure is an epidemic of the 21st century and occurs in 33% of the population above 55 years, with a 30-days-mortality of >11%. Heart transplantation is gold standard for end-stage heart insufficiency, but donor hearts cover only <0.5% of the demand. Alternatives are mechanical heart assist systems (e.g. ventricular assist devices, LVADs) during the drug-supported regeneration phase of the patient's heart.

However, LVADs struggle with device-induced thrombus formation due to inadequate blood flow dynamics of the blood pump rotor, leading to the definition of the 4DbloodROT project. Focus is the R&D on stereolithography-based additive manufacturing, novel hemocompatible materials and surface functionalization, all-together strongly extending the freedom for blood-flow-compatible design of rotors with novel exceptional biomimetic complexity (in 3D-shape & stiffness (=4D)). Final result is an in vitro optimized rotor design as basis for extended in vivo and clinical testing.

Acknowlegement

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Abstract **#1223**

MANIPULATING THE MATRIX: USING NATURE'S TOOLKIT TO INSTRUCT CELL RESPONSES FOR PERCUTANEOUS DEVICES

Nicholas G Fischer$_P$[1], David A De Jong[1], Conrado Aparicio[1]

[1] *University of Minnesota*

Corresponding author's email: Fisc0456@umn.edu

Native extracellular matrixes act as signaling repositories to regulate cell behavior and function. Installation of medical devices disrupts these matrices and interactions of resident cells with microenvironmental cues. As a result, percutaneous devices, medical device with a skin penetrating element such as catheters and dental implants, have suffered high failures rates and infection since 2,500 BCE. Here, we present a new, broadly-applicable physicochemical strategy to leverage matrix for instructing epithelial cell behavior toward extending the lifespans of percutaneous devices.

We first synthesized a series of photopolymerizable polymers with catechol and structurally-similar moieties, easily coated on percutaneous device surfaces, with systematically varied physicochemical properties but similar mechanical properties. Tuning surface charge and polarity enabled us to control keratinocyte hemidesmosome formation, adhesive structures that anchor cells to matrix, formation and downstream signaling. A series of inhibitor and knock-down experiments demonstrated the bioinstructive effect of our materials on elaborated matrix, laminin332 in particular, in an integrin-dependent manner. Finally, we challenged our engineered materials in a course of experiments, including ex vivo, meant to reflect the harsh environment experienced during clinical therapeutic use and showed our materials retain their mechanical and bioinstructive properties. We propose our biostructure polymers direct cell responses via modulation of laminin332's structure in the pericellular matrix. Our strategy leverages the secreted matrix, via material properties, for control of matrix-mediated signals toward enhancing biological responses critical for long-term stability of percutaneous medical devices.

Abstract **#1224**

MULTI-MODAL LABEL-FREE MICROSCOPY TO STUDY STEM CELL REPROGRAMMING IN VITRO IN 3D NICHOID SCAFFOLDS

Valentina Parodi[1], Emanuela Jacchetti[P][1], Arianna Bresci[2], Benedetta Talone[2], Roberto Osellame[3,2], Giulio Cerullo[3,2], Dario Polli[3,2], Manuela Teresa Raimondi[1]

[1] *Department of Chemistry, Materials and Chemical Engineering*

[2] *Department of Physics*

[3] *National Research Council-Institute for photonics and nanotechnologies*

Corresponding author's email: emanuela.jacchetti@polimi.it

We developed a 3D in vitro model for mesenchymal stem cells (MSCs) differentiation based on the Nichoid scaffold, a synthetic microniche with a precise geometry, which emulates the tensional states of the native niche. The hypothesis is that the Nichoid shapes cell morphology thus varying the nuclear pore permeability to transcription factors [1]. In this work, fluorescence microscopy and multimodal label-free microscopy [2] were applied to investigate MSCs response to differentiation media inside 3D Nichoids.MSCs were cultured with adipogenic and chondrogenic culture media for 21 days inside Nichoids and investigated with fluorescence microscopy and label-free multi-photon microscopy at different time points. Results showed that MSCs expanded in the 3D environment and conditioned towards the chondrogenic lineage produced smaller collagen-I deposits with respect to flat substrates. Fibrils were more evident in scaffolds corridors than inside the pores. Contrarily, adipogenesis was enhanced inside Nichoids with respect to controls. The use of multimodal label-free microscopy allowed to chemically identify and spatially localize collagen and lipids in 3D.Our results suggest that the Nichoid stiffness significantly promote adipogenic differentiation, while limiting chondrogenesis.This work received founding from the European Research Council (ERC projects NICHOID, No. 646990, NICHOIDS, No. 754467, MOAB, No. 825159); European Commission (FET-OPEN project IN2SIGHT, G.A. 964481); European Space Agency (ESA project NICHOID-ET, G.A. 4000133244/20/NL/GLC); National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs projects MOAB, G.A. NC/C01903/1 and NC/C019201/1); Italian Ministry of University and Research (MIUR-FARE project BEYOND, G.A. R16ZNN2R9K).

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Abstract **#1226**

COMPARISON OF MICROBIAL PROFILES AND IMMUNOPROFILES IN PATIENTS WITH DENTAL IMPLANTS WITH AND WITHOUT PERI-IMPLANTITIS

Miljana Bacevic[P][1,2], Mariliis Jaago[3,4], Kaia Palm[3,4], Nihal Engin Vrana[5,6], France Lambert[1,2]

[1] *Department of Periodontology and Oral Surgery, University of Liège, Liège, Belgium*

[2] *Dental Biomaterials Research Unit, University of Liège, Liège, Belgium*

[3] *Protobios LLC, Tallinn, Estonia*

[4] *Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia*

[5] *SPARTHA Medical, Strasbourg, France*

[6] *INSERM UMR 1121, Strasbourg, France*

Corresponding author's email: miljana.bacevic@chuliege.be

Introduction: Dental implants have become a well-accepted treatment modality for the replacement of missing teeth with high success rates. However, complications such as peri-implantitis still affect a significant number of patients. As peri-implantitis is biofilm-related, microbiological profiles of subjects may be indicative of future potential for development of this complication and help to develop personalised, surface modifications.

Aim: To perform a clinical study to establish potential similarities and differences in the microbial profiles and antibody-mediated immune response profiles of patients with 'healthy' implants and those affected by peri-implantitis.

Methods: Patients with dental implants were separated into 2 groups: patients with 'healthy' implants and patients with peri-implantitis. Along with clinical data, the following samples were collected: whole blood, saliva, supragingival plaque, and subgingival plaque around implants. Blood plasma was subsequently processed for Mimotope Variation Analysis (MVA) used for immunoprofiling of antibody immune response, while polymerase chain reaction (PCR) method was used for detection and quantification of 10 periodontopathogen DNAs from saliva, supragingival and subgingival plaque.

Results: Statistically significant differences between bacterial loads were only observed in subgingival samples. MVA immunoprofiling revealed differential immune response to mimicking epitopes of Actinomyces and Porphyromonas antigens specifically in subjects with peri-implantitis. When combined, microbial and immunoprofiles identified differences in immune response among subjects with dental implants.

Conclusions: Correlative patterns observed between microbial and immunoprofiles might be further used to highlight the specific immunologic features of host immune response pre-implantation as well as for personalized risk assessment for complication development and mitigation with specific surface modifications.

Keywords

Dental implants; Peri-implantitis; Microbial and immunoprofiles

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Abstract **#1227**

ASSOCIATION OF VNTR RS33966823 POLYMORPHISM IN 3-UTR REGION OF FGFRL1 GENE WITH THE RISK OF COLORECTAL CANCER

Ali fatahian-kelishadrokhi[P][1], Parisa mohammadinejad[1]

[1] *Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*

Corresponding author's email: fatahian1993@gmail.com

Introduction: One of the most common cancers in the world is colon cancer. One of the factors that affect colon cancer is inflammation due to increased cryoprotein protein function, the gene encoding this protein in human FGFRL1. If this gene is damaged, it will increase the performance of cryoprotein protein, and this increase in performance will in turn produce cytokines called IL-1β and inflammation. Regarding the important role of FGFRL1 gene in colon cancer, in this study, VNTR polymorphism was evaluated in healthy and colon cancer patients.

Methods: In this study, 98 patients with colon cancer and 104 healthy subjects selected for VNTR polymorphism of the CIAS1 gene with PCR and gel electrophoresis were studied. Data were analyzed by SPSS 21 software, 2 nd test and logistic regression test.

Results: The results showed that 15 different genotypes and 7 types of alleles were found in the VNTR polymorphism of the FGFRL1 gene. The individuals with the 12-unit repeat allele had the highest and lowest values for those without this allele. The results showed that the genotype lacking a 12-repeat allele compared to the genotype of people with a 12-repeat allele reduced the risk of colon cancer.

Discussion: The results were consistent with the studies (P = 0.01) and the VNTR polymorphism of the FGFRL1 gene can be used as a biomarker for diagnosis of colon cancer, and it regulates various processes related to inflammatory response and colon protection from injury.

Keywords

VNTR; polymorphism; colon cancer

Abstract **#1229**

EVALUATION OF A TISSUE-ENGINEERED NERVE CONDUIT FOR PERIPHERAL NERVE REGENERATION OF A RAT SCIATIC NERVE DEFECT

Alexane Thibodeau[P][1,2], Todd Galbraith[1], Chantal Fauvel[1], Hélène Khuong[1,2], François Berthod[1,2]

[1] *LOEX, centre de recherche du CHU de Québec-Université Laval, Québec (Québec), Canada*

[2] *Département de Chirurgie, Faculté de médecine, Université Laval, Québec (Québec), Canada*

Corresponding author's email: alexane.thibodeau.1@ulaval.ca

Introduction

Treatments for peripheral nerve transections remain a challenge. Nerve damage can lead to severe loss of sensibility and motor functions with important impact on patient's quality of life. Our goal is to produce a scaffold-free nerve tube (NT) prepared exclusively from the patient's own cells. A viable conduit pre-seeded with endothelial (ECs) and Schwann cells (SCs) could allow a faster recovery for patients with major peripheral nerve transections.

Methods

NT's are made of human fibroblast sheets seeded with both EC and SC's and rolled to form a compact tubular structure. NT's were implanted in rats for 22 weeks to repair a 15 mm sciatic nerve defect. Graft innervation was observed by immunofluorescence and toluidine blue staining. The gastrocnemius muscle weight was used as a reliable measurement of reinnervation status. The horizontal ladder rung test was used to determine functional outcome assessment. The sciatic functional index (SFI) was determined from treadmill walking.

Results

The NT enhanced nerve fiber migration. Toluidine blue staining revealed mature myelinated fibers after 22 weeks. Equivalent muscle mass was observed in grafted NT's vs gold standard control autograft. After 22 weeks, a partial motor function recovery was observed in rats with similar efficiency in both NT and autograft ($p>0.05$).

Conclusions

A pre-vascularized NT with autologous cells increases nutrients and oxygen supply through the rapid establishment of a capillary network. Primary SC's may release nerve regeneration factors. In a long-gap model, it has been shown that pre-vascularized and pre-seeded NT's allows for a better motor function recovery.

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Abstract **#1230**

DEVELOPMENT OF A 3D PRINTABLE SOL-GEL SILICA BASED HYBRID INK FOR BONE REGENERATION

Raquel Rodriguez-Gonzalez$_P$[1], Luis Maria Delgado[1], Elia Bosch-Rue[1], Maria Merino-Gomez[1], Leire Diez-Tercero[1], Roman A. Perez[1]

[1] *Bioengineering Institute of Technology, Universitat Internacional de Catalunya*

Corresponding author's email: rrodriguezg@uic.es

The use of 3D printing has enabled the production of custom made scaffolds for tissue regeneration. The success of the technology is based on the meticulous design of inks that offer tunable reactions for the precise printing of scaffolds. In this study, a printable sol-gel silica-based hybrid ink for bone regeneration was developed by combining tetraethyl orthosilicate (TEOS) and gelatin, crosslinked with (3-Glycidyloxypropyl)trimethoxysilane (GPTMS), which was incorporated to reduce the brittleness of the silica and to enhance degradation rates. The sol-gel reaction speed was controlled by using different pHs and reagent ratios to obtain a proper printing window at 35ºC, which would allow the incorporation of bioactive molecules. Moreover, the silica-based ink has to maintain the shape once dried. Therefore, different aging times and drying methods were evaluated to determine the most adequate approach. Preliminary results show that, out of all of the assessed combinations, four have an acceptable printability and shape fidelity. These inks were characterized in terms of printing diffusion rate, collapse area factor, contraction caused by the drying step, and the physicochemical properties. Cell cultures demonstrated that aging time had an effect on cell viability, being cytotoxic for short aging times. To improve the cell behavior, a gelatin coating was applied to the printed scaffolds with the purpose of blocking the unreacted crosslinker and, consequently, reducing its cytotoxicity and improving cell adhesion and proliferation. Therefore, this sol-gel silica-gelatin hybrid ink can be a potential material for the 3D bioprinting of custom made scaffolds for bone regeneration.

Keywords

Silica; 3D-Printing; Bone regeneration

Abstract **#1231**

ESTABLISHMENT OF A TISSUE-ENGINEERED MODEL FOR SKELETAL MUSCLE INJURIES AND DISEASES

Janine Tomasch[P][1,2], Babette Maleiner[1,2], Heinz Redl[3,2], Christiane Fuchs[1,2,4], Andreas Teuschl[1,2]

[1] *Department of Life Science Engineering, UAS Technikum Wien, Vienna, Austria*

[2] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[3] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Vienna, Austria*

[4] *Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, United States*

Corresponding author's email: janine.tomasch@gmx.at

Models adequately mimicking skeletal muscle injuries and diseases are important tools to understand and study mechanisms underlying initiation and progression of diseases as well as tissue regeneration and remodeling after injuries [1]. While a small number of 3D systems for investigating myogenic diseases are already published, there is still a lack of models for myogenic injuries.

Herein, we present how an established tissue engineering approach to create biomimetic skeletal muscle-like tissue can be used to model injuries. Healthy tissue constructs were created through mechanical stimulation of murine myoblasts with a bioreactor [2] and subsequently subjected to different types of stress (e.g. mechanical overstimulation with high frequency or high tensile stress or complete deprivation of mechanical stimulation). Mechanical overstimulation resulted in an immediate stress response as well as prolonged signaling effects (a.o. activation of p38 and ERK 1/2 after 15 minutes, FAK after 6 hours, AKT and S6 after 3 days of stress). Furthermore, high frequency overstimulation led to increased mRNA expression of markers of proliferation (e.g. CCND1) and early- and mid-stage myogenesis (e.g. MyoD, MyoG), and changed morphology of myotubes (random orientation with a hypertrophic phenotype). Deprivation of mechanical stimulation for seven days led to severely impaired integrity of myotubes on a morphological level, but also to downregulation of signaling pathways involved in stress response and myogenic development. Thus, we conclude that the first steps towards the establishment of a biomimetic tissue-engineered skeletal muscle injury model were successful despite further refinement and investigation of the mechanisms involved are still needed.

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Abstract **#1234**

DIFFERENTIAL EFFECTS OF SUBSTRATE STIFFNESS AND ARCHITECTURE ON PROLIFERATION AND DIFFERENTIATION OF HUMAN AND MURINE MYOBLASTS

Janine Tomasch[P][1,2], Babette Maleiner[1,2], Xavier Monforte Vila[1,2], Philipp Heher[2,3], Heinz Redl[2,3], Andreas Teuschl[1,2], Christiane Fuchs[1,2,4]

[1] *Department of Life Science Engineering, UAS Technikum Wien, Vienna, Austria*

[2] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[3] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology/AUVA Research Center, Vienna, Austria*

[4] *Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, United States*

Corresponding author's email: janine.tomasch@gmx.at

Fibrin hydrogels have proven highly suitable scaffold materials for skeletal muscle tissue engineering (SMTE) approaches in the past. Certain parameters of those types of scaffolds, however, greatly affect cellular mechanobiology and therefore the myogenic outcome [1]–[3]. The aim of this study was to identify the influence of scaffold stiffness and architecture on myoblasts and evaluate if those effects differ between murine and human cells. Therefore, myoblasts were cultured on fibrin-coated well-plates (“2D”) or embedded in fibrin hydrogels (“3D”) with different rigidities.

Firstly, we established an almost linear correlation between hydrogels´ fibrinogen concentrations and Young´s moduli in the range of 7.5 mg/ml to 30 mg/mL fibrinogen (corresponds to a range of 7.7 kPa to 30.9 kPa). The effects of substrate stiffness on myoblast proliferation changed depending on hydrogel architecture with an inhibitory effect of higher fibrinogen concentrations in 3D gels and vice versa in 2D. The opposite effect was evident in differentiating myoblasts as shown by expression analysis of myogenesis marker genes and changed myotube morphology. Furthermore, cultivation in a 3D environment slowed down proliferation compared to 2D, with a significantly more pronounced effect in human myoblasts. Differentiation potential was also substantially impaired upon incorporation into 3D gels in human, but not in murine, myoblasts.

With this study, we gained further insight in how scaffold stiffness and architecture affect cellular behavior and myogenic outcome of SMTE approaches. Furthermore, the results highlight the need to adapt parameters of 3D cultivation set-ups established for murine cells when applied for human cells.

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Abstract **#1235**

KERATICNOCYTE-SPECIFIC ADHESION PEPTIDE COATINGS: A NON-ANTIMICROBIAL STRATEGY TO PREVENT INFECTION OF PERCUTANEOUS DEVICES

Conrado Aparicio[P][1], Nicholas G. Fischer[1], Alexandra C. Kobe[1], John Pizarek[1], Jiahe He[1], David A. De Jong[1]

[1] *University of Minnesota*

Corresponding author's email: apari003@umn.edu

Percutaneous devices are widely used medical devices, yet infections lead to a multi-billion dollar healthcare burden(1). The tooth, a Nature's successful percutaneous organ, displays marked longevity/lack of infection because of a unique basement membrane surrounding it that mediates a durable soft tissue attachment to the tooth that prevents access of bacteria and thus, prevent biofilm formation(2). Resident keratinocytes form hemidesmosomes (HDs) that anchor cells to the tooth surface and secures the peri-organ seal(3). However, fibroblast, which cannot form HDs and thus a sound peri-implant seal, can colonize device surfaces contributing to device failure. Coatings of keratinocyte adhesion peptides (KAPs) capable of inducing HD formation and favor proliferation of keratinocytes vs fibroblasts may serve as one strategy for enhanced lifespans of infection-prone percutaneous devices.

Four KAPs curated from basement membrane biomolecules(4) were immobilized using click chemistry and physiochemically characterized. Keratinocyte proliferation, HD formation and downstream formation, and cytokine production were characterized. Two KAPs showed a marked ability to upregulate HD formation and downstream signaling and produce a broader array of wound-repair cytokines compared to other KAPs and controls. Further experiments were carried with these two leading KAPs. Fibroblast proliferation and a fibroblast/keratinocyte co-culture were used to demonstrate cell-specificity and significantly increased expression of proteins related to tooth longevity. Finally, the KAP coatings' durability under simulated use was assessed. Our KAPs coatings represent an alternative to antimicrobials toward enhancing the hosts' ability to prevent infection through sound and durable soft tissue attachment to percutaneous devices.

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Abstract **#1236**

OVINE COLLAGEN TYPE I A POTENTIAL MEDICAL DEVICE FOR RAPID TREATMENT OF WOUND MANAGEMENT: THE POSSIBLE CHALLENGES AND OBSTACLES IN MALAYSIA

Mh Busra Fauzi[1], Ruszymah Bt Hj Idrus[1]

[1] *Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia*

Corresponding author's email: fauzibusra@ukm.edu.my

Total skin loss is a common skin complication affecting millions of people worldwide, mainly in chronic disease. A slight delay in wound management resulted in loosing skin stability and integrity that could lead to chronic wounds or even death. Biomaterial is the main core element in tissue engineering triad to develop a related product such as skin tissue engineering. The ovine collagen type I (OCT-I) was a natural-based biomaterial produced from raw tendon through an acid-based extraction method to obtain high-yield collagen content. Recently, the OCT-I has been proven and tested tremendously since 2011 until now covering the in vitro, in vivo, safety evaluation and pre-clinical model [1-5]. The findings of these evaluations demonstrated positive and promising output to further explore OCT-I as a potential rapid treatment for future use in skin wound management. The next challenging stage is currently faced up covering the up-scaling, quality control and sustainability of OCT-I from laboratory scale to larger production. It requires a lot of time due to involve various parties including government, industrial partner and related regulatory body to make it happened. The Medical Device Authority (MDA), Ministry of Health, Malaysia is a regulatory body responsible to register any medical devices in Malaysia. Specific requirements should be followed through prior to registering different types of medical devices (Class A-D). Therefore, the future driven of successfully commercialized tissue engineering products are depending on the mutual collaboration of academicians, scientists, industrial partners and regulatory bodies in a particular country.

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Abstract **#1237**

REGULATION OF CORNEAL WOUND HEALING BY THE WNK1 KINASE IN A HUMAN TISSUE-ENGINEERED CORNEA

Pascale Desjardins$_P$[1,2], Camille Couture[1,2], Lucie Germain[1,2], Sylvain Guérin[1]

[1] CUO-Recherche, Médecine Régénératrice, Centre de recherche du CHU de Québec, Hôpital du Saint-Sacrement; Département d'ophtalmologie, Faculté de médecine, Université Laval

[2] Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Médecine Régénératrice, Centre de recherche du CHU de Québec, Hôpital de l'Enfant-Jésus; Département de Chirurgie, Faculté de médecine, Université Laval

Corresponding author's email: pascale.desjardins.1@ulaval.ca

INTRODUCTION: Damage to the corneal epithelium triggers important changes in the composition of the extracellular matrix to which the basal human corneal epithelial cells (hCECs) attach. These changes are perceived by integrins and lead to re-epithelialization of the injured epithelium. Among the intermediates downstream of integrin signalling, WNK1 is the kinase whose activity is the most strongly increased during corneal wound healing. The goal of the present study was to determine the contribution of WNK1 to corneal wound healing and to identify the mechanisms and proteins through which WNK1 mediates its influence on that process. METHODS: A scratch wound assay conducted on hCECs grown as monolayers and a wound healing protocol performed on tissue-engineered human corneas (hTECs) were both used as in vitro models to study the impact of a WNK1 pharmacological inhibitor, WNK463, on corneal wound healing process. Gene profiling and transcription factors microarray analyses were also conducted either in the presence or absence of WNK463. RESULTS: Inhibition of WNK1 significantly reduced the rate of corneal wound closure in monolayer and in our 3D model. It also prevented the activation of its downstream target proteins SPAK and OSR1. In addition, WNK1 inhibition also modified the gene expression pattern in hCECs and considerably reduced the activity and expression of a number of key transcription factors in vitro. CONCLUSION: These results will validate a new function for the WNK1 kinase in corneal wound healing and might lead to the identification of a new therapeutic target in the field of corneal wounds.

Keywords

Cornea; Wound healing; WNK1 signaling pathway

Abstract **#1238**

A SOFT ZWITTERIONIC HYDROGEL COATING OF A POLYIMIDE SURFACE TO MINIMIZE THE FOREIGN BODY REACTION TO INTRANEURAL ELECTRODES

Manuele GoriP[1], Sara Maria Giannitelli[2], Rocco Papalia[1], Gianluca Vadalà[1], Loredana Zollo[3], Alberto Rainer[2], Vincenzo Denaro[1]

[1] Laboratory for Regenerative Orthopaedics, Department of Orthopaedic Surgery and Traumatology, Universita' Campus Bio-Medico di Roma (UCBM), Rome, Italy

[2] Laboratory of Tissue Engineering, Department of Engineering, Universita' Campus Bio-Medico di Roma (UCBM), Rome, Italy

[3] Research Unit of Advanced Robotics and Human-centred Technologies, Department of Engineering, Università Campus Bio-Medico di Roma (UCBM), Rome, Italy

Corresponding author's email: m.gori@unicampus.it

Invasive electrodes implanted in peripheral nerves are neural prosthetic devices that can be harnessed to control neuroprostheses in human amputees(1). The Foreign Body Reaction (FBR) is a nonspecific inflammatory response of the host immune system to the implanted material, which represents one of the main challenges to overcome in chronic implants(2). This process is characterized by 3 phases, ending in device encapsulation, due to collagen deposition around the implant, which forms a fibrotic capsule that can ultimately isolate the electrode surface from the surrounding tissue leading to its progressive loss of functionality(2,3).

In this in vitro work, we tested the biocompatibility and ultra-low fouling features of the organic polymeric coating – zwitterionic sulfated poly(sulfobetaine methacrylate) (SBMA) hydrogel – compared to the synthetic coating – poly(ethylene glycol) (PEG) – to reduce plasma protein adsorption and cell adhesion to polyimide surfaces, which are early hallmarks of the FBR(2).

After its synthesis, the SBMA hydrogel was preliminarily characterized through the measurement of the Young's modulus and water contact angle. Cytotoxicity analysis of the zwitterionic hydrogel showed its biocompatibility. In vitro assays with human myofibroblasts and macrophages on PEG- and SBMA hydrogel-coated surfaces were performed to evaluate their adhesion and viability. Because of the high hydration, biocompatibility and swelling capacity, low stiffness, and ultra-low fouling characteristics of the hydrogel, this polymer showed lower cell adhesion, activation and different cell morphology compared to adherent controls.

Overall, our soft SBMA hydrogel could outperform PEG coatings as more suitable dressing of intraneural electrodes for reducing the FBR.

Keywords

Intraneural electrodes; Foreign Body Reaction; Zwitterionic Hydrogel

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Abstract **#1240**

OSTEO-IMMUNE RESPONSES TO THE ENAMEL MATRIX DERIVATIVE COATING LAYER ON TITANIUM-BASED IMPLANTS

Maryam Rahmati$_P$[1], Håvard Jostein Haugen[1], Ståle Petter Lyngstadaas[1]

[1] *Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, 0317 Oslo, Norway*

Corresponding author's email: maryam.rahmati@odont.uio.no

Surface modification of implant surfaces has been a promising solution to avoid the failure of biomaterials after implantation. Although researchers suggest several strategies for surface functionalization of titanium-based implants, only a few studies have compared the osteoimmunomodulatory effects of ionic nanostructures and biofunctionalization in the same biological model. Although enamel matrix derivate (EMD) is known for its positive influences on bone cell responses; its in vivo osteomodulatory responses should be evaluated. In this study, we functionalized the titanium–zirconium implant surface with EMD using an electrochemical cathodic polarization method. Afterward, we evaluated the immune and bone tissue responses to the EMD coated titanium–zirconium implants in the tibia of eight Gray Bastard Chinchilla rabbits. EMD stimulated higher ALP activity and lower cytotoxicity in wound fluid, as well as a lower expression of inflammatory markers after 8 weeks indicating its osteoimmunomodulatory effects after implantation. Overall, the results suggested that implant surface biofunctionalization using EMD could be useful in regulating the immune responses to implant.

Keywords

Enamel matrix derivative; Bone ; Immune system

Abstract **#1241**

3D PLATFORM TO STUDY EPITHELIAL-MESENCHYMAL TRANSITION AND TUMOR CELL MIGRATION IN PANCREATIC CANCER

Claudio Ricci[P][1,2], Edwidge Pugliesi[3], Niccola Funel[3], Serena Danti[4,2]

[1] *National Interuniversity Consortium of Materials Science and Technology, Florence,*

[2] *OtoLab AOUP Azienda Ospedaliera Universitaria Pisana, Pisa, Italy*

[3] *Dept. of Translational Research & New technologies in Medicine & Surgery, University of Pisa, Italy*

[4] *Department of Civil and Industrial Engineering, University of Pisa, Italy*

Corresponding author's email: ricciclaudio09@gmail.com

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the 95% of cancers of the exocrine pancreas and is often associated with a poor prognosis. In addition to therapeutic failures, like chemotherapy, attributed to the PDAC microenvironment, the Epithelial-mesenchymal transition (EMT), is implicated in carcinogenesis and confers metastatic properties on cancer cells improving mobility and invasion. This phenomenon is very important and a new in vitro 3D model is needed in which it can be studied.

Aim: recreate an in vitro 3D model with PDAC primary cells to study the EMT phenomenon and cancer cell migration.

Methods: We fabricated and optimized spongy scaffolds made of PVA/Gelatin (G) (70/30 w/w) via emulsion with G, followed by 1 freezing cycle, and lyophilization. Primary PDAC cells were isolated from patient's specimens, characterized and seeded at 1×106 cells/scaffold. These constructs were cultured for 15 days and assayed at 2, 5, 8, 15 day time-points (n = 3). Along culture time, the AlamarBlue metabolic assay was performed. At each time-point, the constructs were processed for histology: cell morphology was investigated via hematoxylin and eosin staining, proteins involved in the EMT (i.e., EGFR, PAN-CK, TGF-β, MMP-9, actin and desmin) were evaluated via immunohistochemistry (IHC)

Results: IHC results show that the scaffold/cell system resembles the EMT process well, in particular TGF-β protein modulation, that from literature seems to be an important stimulus.

Conclusion/Significance: The PVA/G sponges mimicking well the pancreatic tissue environment, in this way it is possible study the metastatic mechanisms of PDAC through EMT.

Keywords

3D ; cancer ; tissue engineering

Abstract **#1243**

TISSUE HEALING AROUND PRAMA VS. PREMIUM IMPLANTS

Maryam Rahmati[P][1], Håvard Jostein Haugen[1], Mariano S. Alonso[2], Javier S. Esporrin[2], David Palombo[2], Fabio Vignoletti[2]
[1] *Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, 0317 Oslo, Norway*
[2] *ETEP (Etiology and Therapy of Periodontal Diseases) Research Group, University Complutense, Madrid, Spain*
Corresponding author's email: maryam.rahmati@odont.uio.no

Many nnovations are introduced in the macro- and micro-geometry of both the implant necks, abutments, and prosthetic connections, with the aim to promote the maintenance of adequate mucosal height and thickness during healing, minimizing the marginal peri-implant bone loss (1, 2).
Recently, a new implant design has been introduced (Prama, Sweden & Martina, Italy), which is inspired to the biologically oriented preparation technique (BOPT) described for natural teethRecent non-controlled clinical studies, reported favorable results in terms of marginal bone stability and Pink Esthetic Score (PES), at 18 and 36 months, after adopting this implant design in the esthetic area of the upper maxilla (3).
However, a comparative analysis is lacking, both at the clinical and preclinical level, which validates the features of this implant and provides evidence for its efficacy in maintaining stable peri-implant tissues.
The aim of this randomized, controlled, experimental trial was to inquire through a comparative histometric analysis, whether the adoption of the Prama implant design (test), compared to a conventional bone level implant provided with a cilindrical, machined surface abutment (control), could affect the height and thickness of the peri-implant tissues, as well as the position of the free gingival margin (FGM) and of the first bone to implant contact (B).

Keywords
Bone healing; Prama Implant; Soft tissue healing

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Abstract **#1244**

AUTOMATED MONITORING OF SPHEROIDS AND THEIR FUSION KINETICS USING LENS-FREE IMAGING TECHNOLOGY

Thomas Deckers$_P$[1,2,3], Maarten Schoolmeesters[1,3], Jean-Marie Aerts[1,3], Veerle Bloemen[2,3]

[1] *M3-BIORES, KU Leuven, Leuven, Belgium*

[2] *Materials Technology TC, Campus Group T, KU Leuven, Leuven, Belgium*

[3] *Prometheus, Division of Skeletal Tissue Engineering Leuven, KU Leuven, Leuven, Belgium*

Corresponding author's email: thomas.deckers@kuleuven.be

In bottom-up tissue engineering, spheroids are used as building blocks for the fabrication of large tissue structures. During culture, the spheroid size can serve as a quality attribute while their fusion rate is a crucial parameter in biofabrication. Therefore, monitoring technologies with potential for integration and upscaling have to be investigated. In this study, a lens-free imaging (LFI) device was evaluated for automatically monitoring the abovementioned characteristics. Human periosteum derived cells (hPDCs) were seeded onto agarose microwells and cultured up to 1 week in chondrogenic media to form µTissues. After 24 hours, spheroids were captured with bright-field (BF) microscopy and LFI. The spheroids were manually segmented and their area, major axis length and roundness were extracted for validation of the LFI set-up. After 1 week of maturation, a fusion assay was performed in the presence (20 µM) and absence of ROCK-inhibitor for both set-ups. The morphological changes were captured over 24 hours with a 10-minute time interval. For the LFI images, a random forest classifier was trained to segment the doublets and features such as their area and roundness were extracted. The BF images were processed using a thresholding approach. For the LFI set-up, the relative errors on the area, major axis length and roundness were 5.8 ± 4.9%, 4.7 ± 2.5% and 4.7 ± 3.1%, respectively. The classifier obtained a sensitivity, precision and F1-score of respectively 0.959, 0.952 and 0.956. Preliminary analysis of the roundness indicated slower fusion in the absence of ROCK-inhibitor, with consistent results for both set-ups.

Keywords

Lens-free imaging; Spheroids; Monitoring

Abstract **#1245**

INCORPORATION OF LAPONITE®-PNIPAM HYDROGEL INTO BIOACTIVE GLASS SCAFFOLDS FOR BONE REGENERATION

Ronak Janani$_P$[1], Christine C. Le Maitre[2], Chris Sammon[1]

[1] *Materials and Engineering Research Institute, Sheffield Hallam University, Howard St, Sheffield, S1 1WB, UK*

[2] *Biomolecular Sciences Research Centre, Sheffield Hallam University, Howard St, Sheffield, S1 1WB, UK*

Corresponding author's email: ronak.janani@shu.ac.uk

Novel biomaterials are constantly being developed to facilitate regeneration of lost bone tissue resulting from trauma or degenerative diseases. Hydrogel composites are of particular interest in this field due to their therapeutic efficacy and reasonable mechanical strength. We have previously developed a non-biodegradable Laponite® crosslinked pNIPAM-co-DMAc hydrogel which promotes osteogenic differentiation of human mesenchymal stem cells (hMSCs) when loaded with hydroxyapatite nanoparticles1. However, for load-bearing bone defects such hydrogel composites are not adequate mechanically. This study explores the suitability of the Laponite®-pNIPAM-co-DMAc hydrogel incorporated in a bioactive scaffold for bone tissue regeneration. This investigation reports the effects associated with incorporation of the hydrogel on the degradation rate of bioactive scaffolds fabricated via foam replication. This investigation also explores the application of the hydrogel as a means to deliver and maintain hMSCs. The XRD data confirmed in vitro apatite mineralisation after 14 days of aging in PBS for scaffolds with and without hydrogel. Using SEM and EDX, it was shown that the cells' shape and composition were maintained in the hydrogel-glass ceramic system and calcium-rich extracellular matrices were deposited on the cells after 21 days. We are currently evaluating cell viability using a series of histological and immunohistochemical characterisations. We speculate that the hydrogel provides a suitable environment for the cells to survive whilst the bioglass scaffold degrades and promotes cell adhesion and differentiation. We further hypothesise that the non-biodegradable nature of the hydrogel will provide long-term mechanical support whilst the bioactive ceramic network degrades, and a new tissue is generated.

Keywords

Bioactive glass; hydrogel; stem cells

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Abstract **#1246**

OPTIMIZATION OF DOUBLE-CROSSLINKING OF ENZYMATICALLY STABLE COLLAGEN SCAFFOLDS USING SODIUM METABISULFITE AND SODIUM BOROHYDRIDE TO CONTROL CYTOTOXICITY

Mohammad Mirazul Islam[P][1], Claes H. Dohlman[1], James Chodosh[1], Miguel Gonzalez-Andrades[1,2]

[1] Massachusetts Eye and Ear and Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

[2] Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Department of Ophthalmology, Reina Sofia University Hospital and University of Cordoba, Cordoba, Spain

Corresponding author's email: mirazulislam@yahoo.com

Collagen scaffolds, one of the most used biomaterials in corneal tissue engineering, are usually crosslinked to improve mechanical properties, enzyme tolerance capacity, and thermal stability. More biocompatible crosslinkers, such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), provide lower crosslinking density and reduced mechanical properties. Conversely, those that generate mechanically more robust scaffolds, such as glutaraldehyde (GTA), tend to induce greater toxicity. Herein, we evaluate the capability of sodium metabisulfite (SM) and sodium borohydride (SB) to restore biocompatibility after crosslinking.

EDC-crosslinked collagen scaffolds were treated with different concentrations of GTA. To mask the free aldehyde group produced through the GTA crosslinking, scaffolds were then treated with SM or SB. Mechanical and functional properties were evaluated. Cell viability was studied using different cultured corneal cell types. The effect of each scaffold treatment on human monocyte differentiation was evaluated. One-way ANOVA was used for statistical analysis.

EDC-GTA double crosslinking significantly improved the mechanical properties and enzymatic stability of the collagen scaffold. GTA decreased cell biocompatibility, but this was reversed by treatment with SB or SM. SB or SM did not reduce or affect mechanical properties, enzymatic stability, or transparency of the double crosslinked scaffold. Differentiation of monocytes to activated macrophages was not initiated in contact with any scaffold.

Our result demonstrated that GTA improves mechanical properties of the EDC crosslinked scaffolds in a dose dependent manner, and that subsequent treatment with SB or SM restores biocompatibility. This novel manufacturing approach would facilitate the translation of collagen-based artificial corneas to the clinical setting.

Abstract **#1247**

NATURAL POLYPHENOLS QUERCETIN AND HYDROXYTYROSOL AS EFFECTIVE INHIBITORS OF HEPATIC STEATOSIS: A NAFLD-ON-A-CHIP STUDY

Manuele Gori[1], Sara Maria Giannitelli[2], Andrea Zancla[3,2], Pamela Mozetic[4], Marcella Trombetta[2], Nicolò Merendino[5], Alberto Rainer[2,6,7]

[1] *Laboratory for Regenerative Orthopaedics, Department of Orthopaedic Surgery and Traumatology, Università Campus Bio-Medico di Roma (UCBM), Rome, Italy*

[2] *Laboratory of Tissue Engineering, Department of Engineering, Università Campus Bio-Medico di Roma (UCBM), Rome, Italy*

[3] *Università degli Studi Roma Tre, Rome, Italy*

[4] *International Clinical Research Center, St. Anne's University Hospital, Brno, Czechia*

[5] *Department of Ecological and Biological Sciences, Loc. Riello s.n.c., Università della Tuscia, Viterbo, Italy*

[6] *Institute of Nanotechnology (NANOTEC), National Research Council, via Monteroni, Lecce, Italy*

[7] *Interuniversity Center for the promotion of the 3Rs principles in teaching and research, Italy*

Corresponding author's email: m.gori@unicampus.it

Organs-on-chip (OoCs) are catching on as a promising and valuable alternative to animal models, in line with the 3Rs principle. OoCs enable the creation of 3D tissue microenvironments with pathophysiological relevance at unparalleled precision and complexity, offering new opportunities to model human diseases in vitro and to test the potential therapeutic effect of novel drugs, while overcoming the limited predictive accuracy of conventional 2D culture systems (1,2,3). Here, we present a liver-on-a-chip platform to investigate the effects of two plant-derived polyphenols, namely Quercetin and Hydroxytyrosol, on nonalcoholic fatty liver disease (NAFLD) using a high-content analysis readout methodology (4,5). NAFLD is currently the most common cause of chronic liver disease worldwide; however, its complex and multifactorial pathogenesis is still far from being clear, and a definitive treatment has not yet been established (6,7). In our experiments, we observed that both polyphenols seem to hamper the progression of the free fatty acid-induced hepatocellular steatosis, showing a cytoprotective effect due to their antioxidant and lipid-lowering properties (8). In conclusion, the findings of the present work could provide novel therapeutic strategies to contrast the onset and progression of NAFLD.

Keywords

Hepatic steatosis; Polyphenols; High-content analysis

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Abstract **#1249**

FLEXIBLE PIEZOELECTRIC PATCHES FOR IMPROVEMENT OF HEART TISSUE ACTIVITY

Luís Miguel Monteiro$_P$[1], Pedro J. Gouveia[2,3], Susana Rosa[4], Francisco Vasques-Nóvoa[5,6,7], Rita Gomes[5,8,9], Ifigeneia Bardi[10], Leonardo Ricotti[11], Rui Cerqueira[7], Adelino Leite-Moreira[7], Filippo Perbellini[12,13], Cesare Terraciano[13], Perpétua Pinto-do-Ó[5,6,9], Lino Ferreira[4,14,15], Diana S. Nascimento[5,6,15]

[1] Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

[2] Tissue Engineering Research Group, Department of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Ireland

[3] Advanced Materials and BioEngineering Research (AMBER) Centre, RCSI, Ireland

[4] Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

[5] I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

[6] INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

[7] Departamento de Fisiologia e Cirurgia Cardiotorácica, Faculdade de Medicina da Universidade do Porto, Rua Doutor Plácido da Costa, 4200-450 Porto, Portugal

[8] INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

[9] ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

[10] National Heart & Lung Institute, Imperial College London, London, W12 0NN, UK

[11] The BioRobotics Institute, Scuola Superiore Sant'Anna, Pontedera, PI Italy 56025

[12] Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hanover, Germany

[13] National Heart and Lung Institute, Imperial College London, Du Cane Road, London W12 0NN, UK

[14] Faculty of Medicine, University of Coimbra, Coimbra, Portugal

*[15] * - co-senior author*

Corresponding author's email: luis.m.monteiro3@gmail.com

Ischemic heart diseases are the leading cause of death worldwide. Novel approaches have been focusing on restoring cardiac function by mainly targeting cardiac repair, however in vivo studies promoting both contractile and electrical functional recovery are scarce. Piezoelectric materials are capable of generating electric current upon mechanical deformation. Since the heart exhibits robust cyclic movements, implantation of these materials on the myocardium holds great potential for designing biomaterials with self-sustained electrical stimulation, harnessing the contractile proficiency of healthy regions of the ventricle to stimulate damaged ones. In this work, piezoelectric patches implanted on ex vivo rat Langendorff-perfused working hearts and cardiac slices did not preclude tissue function and had the ability to slightly improve its contractility, comparing with control electrically inert PCL patches. Following one month of in vivo heart implantation on a mouse model of myocardial infarction, piezoelectric patches caused improvement of the cardiac function, mainly on heart electrical integrity. Despite no notable alteration in histological parameters, the transcriptome of piezoelectric patch-implanted hearts was altered specifically on the more contractile/proficient portion of the left ventricle, showing a downregulation of genes associated with matrix remodeling and heart failure when compared with the PCL control, suggesting a more efficient functional recovery from the infarction by the piezoelectric group. The current work is the first therapeutic cell-free biomaterial-based patch with piezoelectric properties that can be directly applied to the healthy myocardium without compromising its function, while causing an improvement on the overall function of the mouse infarcted heart.

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Abstract **#1250**

FABRICATION AND CHARACTERIZATION OF 3D POROUS SCAFFOLDS FROM AMNIOTIC MEMBRANE IN COMBINATION WITH CHITOSAN/ALGINATE FOR BONE TISSUE ENGINEERING OF ADIPOSED-DERIVED MESENCHYMAL STEM CELLS.

Parisa Taklifi[P][1], Mahboubeh Kabiri[1]

[1] *Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran*

Corresponding author's email: parisataklifi@ut.ac.ir

In the past few decades, many attempts have been made to manufacture an ideal tissue-engineered graft for intricate tissue of bone; however, inconsistencies between in vitro and in vivo experiments still exist due to negligence towards the immune system/implant interactions. Regarding the immunomodulation as a new critical factor in bone tissue engineering (BTE), amniotic membrane (AM)-derived products with its immunomodulatory effects have recently shown promising applications in ocular or cutaneous treatments and have paved the way for other applications such as BTE. Herein, we hypothesized that AM scaffold or a combination of AM, chitosan, and alginate scaffold could enhance osteogenic activity and induce angiogenesis in in vivo studies. We aimed to exploit human AM matrix to fabricate a 3D porous scaffold allowing cellular infiltration, proliferation, osteogenic differentiation, and in vivo angiogenesis. We reported mechanical and structural properties of the scaffolds and assessed cell viability regarding these groups. As they demonstrated increased cell proliferation, we investigated the osteogenic differentiation and mineral deposition of adipose-derived mesenchymal stem cells within scaffolds in the presence and absence of osteogenic supplements. We further analyzed the Balb/c subcutaneous implants of the scaffolds for inflammatory reaction, implant/immune cell interactions, and angiogenesis. Incorporation of amniotic membrane ECM bone biomimetic scaffolds promoted osteogenesis even in the absence of osteogenic factors and induced angiogenesis while reducing chronic inflammation at the site of implantation. Collectively, our data on AM derived composite porous scaffolds stands on being appropriate as bone graft substitutes.

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Abstract **#1251**

HIERARCHICALLY MINERALIZING 3D PRINTED SCAFFOLDS FOR HARD TISSUE REGENERATION

Abshar Hasan$_P$[1,2], Karen Marshall[3], Jonathan Wojciechowski[4], Sherif Elsharkawy[5], David Eglin[6], Richard Oreffo[3], Molly Stevens[4], Alvaro Mata[1,2]

[1] *School of Pharmacy, University of Nottingham, Nottingham, UK*

[2] *Department of Chemical and Environmental Engineering, University of Nottingham, Nottingham, UK*

[3] *Bone & Joint Research Group, Centre for Human Development, University of Southampton, Southampton, UK*

[4] *Department of materials & Department of bioengineering, Imperial College London, London, UK*

[5] *Center for Oral, Clinical and Translational Sciences, King's College London, London, UK*

[6] *Polymers Focus Area, AO Research Institute Davos, Davos, Switzerland*

Corresponding author's email: abshar.hasan@nottingham.ac.uk

Hard tissue disorders such as osteoporosis, maxillofacial and cranial defects, oral defects affecting enamel, dentine, and periodontal tissues affect more than half of the world population [1, 2]. Here we report an integrated approach combining additive manufacturing with supramolecular chemistry to develop acellular hierarchically mineralizing scaffolds for hard tissue regeneration. Our approach uses the interplay between protein's order and disorder to generate a supramolecular framework capable of triggering hierarchical apatite growth within 3D printed scaffolds guiding mineralization from the nanoscale up to complex macroscopic structures. Polymeric scaffolds of desired structural parameters such as porosity, pore size, and pore connectivity were 3D printed using computer-controlled rapid prototyping technology. Scaffolds were coated with elastin-like protein (ELP) to form a uniform layer of ~10 µm thickness throughout. The mineralized scaffolds were acid-resistant and exhibited high stiffness and hardness of up to 33.0 and 1.1 GPa, respectively [3]. Furthermore, the scaffolds supported adhesion and proliferation of human bone marrow cells (hBMCs) and stimulated the expression of alkaline phosphatase (ALP) as compared to naïve scaffolds. Overall, this approach integrates advantages of supramolecular chemistry, tunable organic-inorganic interactions, additive manufacturing, and commemorates ease of design and fabrication of a novel platform to enhance osteoinductivity and osteoconductivity in bone regeneration. We envisage that this approach can have important implications for the design of smart biomaterials which not only can acellularly self-mineralize by drawing ions from the implant site but also exhibit the capacity to infiltrate and integrate with the underlying native tissues.

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Abstract **#1252**

DEVELOPMENT OF CARTILAGE DECELLULARIZED EXTRACELLULAR MATRIX INK

Katie Hogan[P][1], Mollie Smoak[1], Antonios Mikos[1]

[1] *Rice University*

Corresponding author's email: kh48@rice.edu

Decellularized extracellular matrix (dECM) has become an attractive material for applications in regenerative medicine and tissue engineering due to its retention of native biochemical cues to promote cell growth, proliferation, and differentiation. Extrusion 3D printing represents a versatile fabrication platform for dECM hydrogel scaffolds due to its precise control over internal microarchitecture and bulk geometry. However, cartilage dECM inks often lack the rheological and mechanical properties for traditional extrusion printing and often require synthetic copolymers or scaffolding. Previously, gelatin-based sacrificial baths (FRESH) have enabled printing of soft hydrogels but demonstrate opposite gelation kinetics compared to dECM. Here, the objective of our work is to develop and characterize a cartilage dECM ink for extrusion printing without the need for additional polymer incorporation using a Pluronic-based sacrificial bath, which demonstrates LCST gelation behavior, and dual-gelation via riboflavin photo-crosslinking.

Harvested hyaline cartilage was decellularized using physical, chemical, and enzymatic means, and biochemical characterization performed to quantify sulfated glycosaminoglycans, collagen, total protein, and DNA content. Rheological and mechanical characterization were performed to evaluate viscosity, shear thinning behavior, temperature sweep, photo-crosslinking, and compressive modulus. Following 3D printing, constructs were evaluated for print resolution via fiber diameter and pore circularity. Additionally, long-term stability and degradation of the constructs were evaluated in collagenase. In addition to enabling cartilage dECM printing without co-polymers or scaffolding, this printing system may be adapted for broader use for dECM and LCST polymer inks which have not traditionally been compatible with extrusion 3D printing.

Abstract **#1253**

A DESIGNER ANTIMICROBIAL PEPTIDE: ROLE OF SUPRAMOLECULAR PEPTIDE ASSEMBLY AND IMPLICATIONS FOR ITS USE AS BUILDING BLOCK FOR POTENT ANTIMICROBIAL BIOMATERIALS

Conrado Aparicio[P][1], Zhou Ye[1], Dhiraj Kumar[1], Alexandra C. Kobe[1], Ting Sang[2], Sergio Acosta[3], Janaki Khot[1]

[1] *University of Minnesota*

[2] *Nanchang University*

[3] *University of Valladolid*

Corresponding author's email: apari003@umn.edu

Selected antimicrobial peptides (AMPs) can self-assemble into supramolecular structures (1), which has been overlooked as causative of their antimicrobial activity as well as a potential tool for developing potent multifunctional antimicrobial biomaterials. Moreover, the higher antimicrobial potency of D-enantiomers compared to L-enantiomers of AMPs cannot always be attributed to their different resistance to protease degradation and enables the development of more effective biomaterials(2). We have vastly studied these effects and the structure-function correlations of a designer AMP, GL13K, derived from the parotid secretory protein(3). We have also developed potent antimicrobial GL13K coatings on medical devices and tissues to protect them against infection for their use in dentistry and orthopedics(4,5).

Here, we tested and compared all L- and D-amino acid versions of GL13K and its negative randomized amino acid peptide versions, GL13K-R. We assessed (a) structural links between the AMP secondary structure, supramolecular self-assembly dynamics, and antimicrobial activity, (b) differences in structural interactions with model bacterial membranes and components of the bacterial outer envelope, and (c) governing surface physicochemical properties to build effective AMP coatings. We demonstrated that (a) the ability of and faster dynamics for supramolecular assembly of D- GL13K compared to L-GL13K was correlated with higher AMP activity, (b) specific and strong interactions between D-GL13K and bacteria-wall proteoglycans was suggestive of higher potency against Gram-positive bacteria, and (c) surface polarity of AMP-coated substrates was the dominant factor governing AMP/substrate interactions. Our findings can instruct the universal design of efficient self-assembled AMP coatings on biomaterials, biomedical devices and/or natural tissues.

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Abstract **#1255**

EVALUATION OF INJECTABLE SODIUM ALGINATE AND GELATIN HYDROGELS FOR TREATMENT OF URINARY INCONTINENCE

Sedigheh Joughehdoust[P][1,2], Hesam Rezaei[3], Azadeh Asefnejad[3], Morteza Daliri-joupari[4], G. van Koeveringe[5,6], S. Ghazanfari[7,8]

[1] *Department of Urology, Maastricht University, The Netherlands*

[2] *Aachen-Maastricht Institute for Biobased Materials (AMIBM), University of Maastricht*

[3] *Department of Biomedical Engineering, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran*

[4] *National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*

[5] *Department of Urology, School for Mental Health and Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands*

[6] *Department of Urology, Maastricht University Medical Center (MUMC+)*

[7] *Aachen-Maastricht Institute for Biobased Materials (AMIBM), University of Maastricht, Urmonderbaan 22, 6167 RD, Geleen, The Netherlands*

[8] *Institute of Applied Medical Engineering, RWTH Aachen University*

Corresponding author's email: s.joughehdoust@maastrichtuniversity.nl

In this study, the injectable sodium alginate and gelatin hydrogels were synthesized and modified by using silane coupling agents and calcium chloride to improve physicochemical and mechanical, the behavior of hydrogels. The morphology of the hydrogels displayed a homogeneous structure for all samples. The chemical structure of the raw materials and the hydrogels confirmed the possible cross-linking reaction. High level of absorption capacity and resistance to rapid degradation demonstrated suitable stability of the gels with the highest concentration of polymers and lowest (3-glycidyloxypropyl) trimethoxysilane (GPTMS): polymer ratio (0.5:1 w/w). Bioactivity evaluation confirmed the formation of hydroxyapatite layers that can improve the effectiveness and durability of the hydrogels. The rheological properties of the hydrogels indicated the higher stability of the storage modulus and viscosity when the higher concentration of polymers and the lower concentration of cross-linker were applied. Consequently, hydrogels (10 %W/V gelatin, 8.5 %W/V sodium alginate, and GPTMS: polymer ratio (0.5:1 w/w)) can be useful for further in vivo and pre-clinical evaluation for urinary incontinence treatment.

Keywords

Urinary incontinence; Injectable hydrogel; Sodium alginate/gelatin

Abstract **#1257**

INVESTIGATION OF ADDING ALCOHOLIC EXTRACT OF ALOE VERA TO ELECTROSPUN GELATIN NANOFIBERS AND IN VITRO STUDY FOR WOUND HEALING APPLICATIONS

Sedigheh Joughehdoust[P][1,2], Ali Manafi[3], Sahar Alidaghi[3], S. Ghazanfari[4,5]

[1] *Department of Urology, Maastricht University, The Netherlands*

[2] *Aachen-Maastricht Institute for Biobased Materials (AMIBM), University of Maastricht*

[3] *Department of Engineering, Shahrood Branch, Islamic Azad University, Shahrood, Iran*

[4] *Aachen-Maastricht Institute for Biobased Materials*

[5] *Institute of Applied Medical Engineering*

Corresponding author's email: s.joughehdoust@maastrichtuniversity.nl

In this study, the possibility of binding of alcoholic extract of aloe vera to the electrospun gelatin was investigated. After producing the gelatin fibers, they were immersed in the aloe vera extract solution to allow the extract to be infiltrated into the fibers. Scanning electron microscopy (SEM) and optical microscopy revealed that the fibers were well oriented on the aligned nanofibers. The 3D cavities between the fibers might be suitable sites for cell migration and loading the antibiotic drugs or plant extracts that release the drug into the nanofibers compared to the films due to a better surface area to the volume of nanofibers. The NIH-3T3 fibroblasts cells were cultured and the cytotoxicity was evaluated. The results of the MTT assay showed cell viability and proliferation of the scaffolds and the aloe vera extract. Gelatin plays an important role in the cell adhesion. To improve the mechanical and thermal properties of gelatin, glutaraldehyde has been used as a cross-linker. The results showed that as the concentration of glutaraldehyde increases, the number of cavities decreases which confirms the enhancing of the scaffold strength. These nanofibers scaffolds might be a good candidate as a dressing material for wound healing applications.

Keywords

aloe vera; gelatin; electrospinning

Abstract **#1258**

THE EFFECT OF PORE ARCHITECTURE ON CELL-ECM FORMATION IN HYDROXYAPATITE BONE SCAFFOLDS IN 3D PERFUSION CULTURE

Ehsan Sadeghian Dehkordp[1,2], Mahtab Asadian[2,3], Joris Bleukx[2,4], Liesbet Geris[1,2,4]

[1] *GIGA In silico medicine, Biomechanics research unit, University of Liège, Belgium*

[2] *Prometheus, the R&D division for skeletal tissue engineering, KU Leuven, Belgium*

[3] *Research Unit Plasma Technology (RUPT), Department of Applied Physics, Ghent University, Belgium*

[4] *Department of Mechanical Engineering, Biomechanics Section, KU Leuven, Belgium*

Corresponding author's email: e.sadeghian@uliege.be

Calcium phosphate-based scaffolds with porous structures similar to natural bone are frequently used in bone tissue engineering. The current challenge to further improve their performance is focusing on the optimization of their 3D structure with additive manufacturing allowing to produce scaffolds with a high degree of local control on the internal pore architecture. This allows to balance the biological requirements with the biomechanical ones, such as is the case for gradient porous scaffolds (GPS) which are porous structures where the porosity changes in space with a specific gradient. To properly test the effectiveness of scaffold architecture on drawing in cells and guiding neotissue formation in vitro, an environment similar to the physiological conditions is needed. In spite of ample studies showing the importance of pore gradient in conventional in vitro models, much less research has been done on the performance of GPS biomaterials in a dynamic 3D culture environment.

Therefore, this study investigated the influence of pore architecture on cell proliferation and matrix deposition. Immortalized bone marrow mesenchymal stem cells were seeded on additive manufactured CaP scaffolds and cultured for up to 21 days in 3D perfusion bioreactor. Live/Dead staining of the construct was performed to show the presence of live cells. Contrast-enhanced nanoCT imaging was used to visualize the neotissue (cells + extracellular matrix) formed inside the scaffold.

This study provides a quantitative insight into the spatial gradient of scaffold's pore architecture and confirms the influence of perfusion bioreactor system for the in vitro development of 3D cell–carrier constructs.

Keywords

Calcium phosphates; Pore architecture; 3D perfusion culture

Abstract **#1259**

MICROCARRIERS WITH COMPLEX ARCHITECTURES MANUFACTURED BY TWO-PHOTON LITHOGRAPHY FOR MECHANOBIOLOGICAL MANIPULATION AND EXPANSION OF MESENCHYMAL STEM CELLS

Jason Hutchinson[P][1], Felicity Rose[1], Cameron Alexander[1], Ricky Wildman[1]

[1] *University of Nottingham*

Corresponding author's email: jason.hutchinson@nottingham.ac.uk

Mesenchymal stem cells (MSCs) have been widely utilised for tissue repair, however for effective treatment large numbers of cells are needed due to low retention in defect sites [1]. Stem cell populations can be expanded through culture on microcarriers. Current strategies struggle to meet population sizes required as the availability of surface area is not the only factor to be considered when promoting cell growth. Complex architectures exhibiting specific biophysical cues can increase MSC expansion capacity and control differentiation [2]. The production of bio-instructive microcarriers using biophysical cues have the potential for in vitro cell expansion that surpasses currently available technologies as well as the capability for in vivo utilisation as a multifunctional tissue modulator through particle-based scaffolds.

Two photon lithography creates highly accurate microscale structures with feature sizes down to 100nm. This technology was employed to create microparticles with varying designs for culture with MSCs. A variety of microcarrier designs were created and fabricated with optimisation of materials, fabrication procedures and post processing strategies. Initial experimentation has provided insight into cellular infiltration, proliferation and migration across microparticle designs highlighting several promising architectures. Useful methodology for directing cells to geometries through pHEMA coated 2PL fabrication has been established and holds much promise for further experimentation. Current work focuses on examination of cell expansion yield for identified designs leading to phenotypic differentiation analysis of MSCs over the expansion period. Finally, novel µSLA technology will be explored to meet clinically relevant scales and compare effectiveness of bio-instructive microcarriers to currently available technologies.

Keywords

Microcarriers; Mesenchymal stem cell; Two photon lithography

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Abstract **#1260**

BIOMATERIAL SCAFFOLD ARCHITECTURE IMPACTS TISSUE VASCUARLIZATION PATTERN VIA MATRIX STRAIN

Gennifer Chiou[1], Aparna Gorthi[2], Joo Ong[1], Yidong Chen[2], Rena Bizios[1], Chris Rathbone[1], Teja Guda[P][1]

[1] *University of Texas at San Antonio*

[2] *University of Texas Health San Antonio*

Corresponding author's email: teja.guda@utsa.edu

Introduction: Vascular network development in volumetric tissue defects remains a challenge in tissue engineering. We propose that scaffold architecture which dictates cell proliferation and tissue morphogenesis, also controls stromal-vessel interactions. We evaluate interaction between mesenchymal stem cells (MSCs) and microvascular fragments (MVFs) within hydroxyapatite (HA) scaffolds of various pore sizes containing fibrin hydrogels. Additionally, a computational model validates the architectural effects on vascularization observed in vitro.Methods: Sintered HA scaffolds were synthesized by template coating at 3 pore sizes (450, 340 and 250 µm). Primary bone marrow MSCs were seeded first at 1x105/scaffold. Fibrin gels were constituted at 2:5 fibrinogen:thrombin at 20 mg/mL. MVFs isolated from rat fat pads were seeded at 20,000 MVFs/mL. The MVF-fibrin filled MSC seeded-HA scaffolds were then cultured for 3, 7, 14 and 21 days in vitro. Decalcified samples imaged (lectin staining) or RNA extracted for sequencing. µCT scans were used to create finite element models of scaffolds for each pore size and AngioFE (FEBio) was used to simulate capillary development over a period of 7 days.Results and conclusions: Lectin staining showed continued vessel growth and branching. Highly porous interconnected scaffolds influence the development of capillary networks: fibrin gels offer limited resistance to capillary morphogenesis, 450 µm pores showed capillary loops within pores, 340 µm pores showed a mix and the 250 µm pores showed vessels bridging across pores. Computational modeling with appropriate inputs showed promise in matching biological experimental findings which can be leveraged for improved porous biomaterials scaffold design.

Abstract **#1261**

TENDON/LIGAMENT REGENERATION USING A SCAFFOLD-FREE BIOFABRICATED 3D CONSTRUCT OF AUTOLOGOUS ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS IN RABBITS.

Daiki Murata[P][1], Ryota Fujimoto[1], Shoko Takao[1], Miho Miyake[1], Anna Maria Nakamura[1], Koichi Nakayama[1]
[1] *Saga University, Center for Regenerative Medicine Research*
Corresponding author's email: daiki_net_official@yahoo.co.jp

Objective: This study was designed to investigate the regeneration of patella tendon/ligament using a scaffold-free biofabricated 3D construct of autologous adipose tissue-derived mesenchymal stem cells (AT-MSCs) in rabbits.
Design: A columnar construct (inner diameter of 2.5 mm, height of 3 mm) was fabricated by using bio-3D printer (Regenova; Cyfuse Biomedical K.K., Tokyo, Japan), and it was consisted of 320 spheroids each containing 2.0 × 10^4 AT-MSCs. After the construct was matured following to fusion of the spheroids, it was cut to patch shape (length of 8 mm, width of 3 mm). And then, it was autologously implanted into a defect at the right patella tendon/ligament in each of eighteen adult Japanese white rabbits (kbt-JW; Biotech Co., Ltd, Tosu, Japan; 2.5-3.5 kg; 27-37 weeks old). The contralateral (left patella tendon/ligament) defect was untreated as a control. The eighteen rabbits were divided into three groups (6, 12, and 24 weeks post-implantation groups), and those patella tendons/ligaments were eventually evaluated histopathologically.
Results & Conclusion: Currently, a difference between the implanted site and the control site was observed in tendon/ligament thickness. For the future, differences between the both sites will be examined in orientation of collagen fibers and network of existing cells. These results will probably suggest that autologous implantation of a scaffold-free biofabricated 3D construct of AT-MSCs into a tendon/ligament defect could regenerate the original structure of tendon/ligament for post-operative 6 months.

Keywords
Tendon/ligament regeneration; Scaffold-free; Biofabricated 3D construct

Abstract **#1262**

KINETICS ON AGGREGATE BEHAVIORS OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN STATIC SUSPENSION AND ROTATING FLOW CULTURES

Akihiro Hashida[P][1], Toshimasa Uemura[2], Masahiro Kino-oka[1]

[1] *Department of Biotechnology, Graduate School of Engineering, Osaka University*

[2] *Department of Precise and Science Technology, Graduate School of Engineering, Osaka University*

Corresponding author's email: hashida_akihiro@bio.eng.osaka-u.ac.jp

Understanding of kinetics on aggregate behaviors of human induced pluripotent stem cells (hiPSCs) is important knowledge for developing stable mass culture process. Here we found critical factors inhibiting growth of hiPSCs in suspension culture through analysis of aggregate size distributions and kinetic parameters when culturing two types of cell lines (253G1 and 201B7 lines) in static suspension and rotating flow cultures, which were realized by multi-dimple plate and rotating wall vessel, respectively. In the case of 253G1 line in rotating flow culture, aggregate number decreased and size increased drastically during culture time, t = 0 - 24 h due to coalescence between cell aggregates. The apparent specific growth rate decreased after t = 24 h although cell number and aggregate size gradually increased in static suspension culture. On the other hand, in the case of 201B7 line in rotating flow culture, cell and aggregate number, and aggregate size kept constant levels during t = 24 - 72 h due to collapse of cell aggregates by stripping of single cells from aggregate, suggesting that specific death rate increased after t = 24 h despite constant levels of apparent specific growth rate and aggregate number in static suspension culture. Distributions of E-cadherin and extracellular matrices in aggregates, which critically affects coalescence and collapse, respectively, were also quite different between cell lines. Our kinetic analysis concluded that excessive increase of aggregate size due to coalescence and stripping of single cells due to collapse critically affected growth of hiPSCs in suspension culture.

Keywords

human induced pluripotent stem cells; kinetics on aggregate behaviors; rotating flow culture

Abstract **#1263**

FIBER ORIENTATION IMPACTS MATRIX DEPOSITION TO ESTABLISH LARYNGOTRACHEAL SCAR MODELS

Solaleh Miar[1], Gregory Dion[1,2], Ryan Branski[3], Joo Ong[1], Rena Bizios[1], Teja Guda$_P$[1]

[1] *University of Texas at San Antonio, Texas, USA*

[2] *United States Army Institute of Surgical Research, Ft. Sam Houston, TX, USA*

[3] *NYU Voice Center, Department of Otolaryngology–Head and Neck Surgery, New York University School of Medicine, New York, New York, U.S.A*

Corresponding author's email: teja.guda@utsa.edu

Introduction: The inner lining of the upper airway includes ciliated epithelium and lamina propria essential for barrier function. This layer is disrupted upon injury and results in inflammation and fibrotic scarring[1]. We developed a model to study the impact of basement architectural cues on the epithelial-fibroblast interaction at air-liquid interface by using randomly-oriented and aligned polycaprolactone (PCL) fibers. Materials and Methods: Plasma treated randomly oriented and aligned PCL electrospun fibers were placed in transwell chambers and were seeded with human tracheal fibroblasts (HTFs) for 7 days and then human bronchial epithelial cells (HBEs) were introduced above the HTF layer. An air-liquid interface was established on day 14 to promote HBE differentiation. Permeability, cell proliferation, and expression of fibroblast (fibronectin and S100A4) and epithelial (MUC5A) markers were evaluated using ELISA and immunofluorescence (IHC) imaging (n=6). Quantitative data were compared using one-way Analysis of Variance (ANOVA) followed by Tukey's test for post hoc determination of significant differences at $p<0.05$.Results and Discussion: Fiber alignment resulted in higher expression of fibroblast markers during the first 7 days while randomly oriented fibers generally caused higher (27%) cell proliferation over time. In addition, IHC images revealed homogenous HBE growth above the HTFs layer with significant laminin-rich matrix deposited at the interface and dispersed spheroidal epithelial clusters observed in both groups. Larger epithelial spheres were observed in coculture on randomly oriented fibers with rudimentary ciliated structures.Conclusions: A successful epithelial-fibroblast coculture system with pro-fibrotic behavior was achieved by controlling architectural cues introduced during initial fibroblast-epithelial interactions.

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Abstract **#1264**

H19-ENRICHED EXTRACELLULAR VESICLES PROMOTE ANGIOGENESIS IN VITRO

Andreia Vilaça$_P$[1,2], Hugo Fernandes[1,3], Leon de Windt[2,3], Lino Ferreira[1,3]

[1] *Faculty of Medicine and Centre for Neurosciences and Cell Biology, University of Coimbra, Portugal*

[2] *Department of Molecular Genetics, Faculty of Science and Engineering, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands*

[3] *Equal contribution*

Corresponding author's email: andreiapvilaca@gmail.com

The expression of H19 long non-coding RNA (lncRNA) is known to steeply diminish during the development of the cardiovascular system. While H19 knockdown has been shown to hinder endothelial cell proliferation and their capacity to form capillary-like structures, approaches aimed at restoring H19 expression have demonstrated controversial results. Here, we used extracellular vesicles (EVs), known to be involved in vascular development, growth and maturation, and modulate their content with H19 in order to analyze their effect on therapeutic angiogenesis. For that purpose, we overexpressed the mouse H19 gene in human mesenchymal stem cells (WJ-MSC) for 48 h, after which we collected and characterized the EVs. Our results showed that H19-transfected WJ-MSCs secreted EVs enriched in H19 without changing the physical-chemical profiles of scramble-transfected EVs. Furthermore, H19-EVs were internalized by mouse aortic endothelial cells (MAECs), which resulted in a >2-fold increase in H19 expression compared to control. Treatment of MAECs with H19-EVs significantly ($p<0.05$) accelerated their angiogenic response, resulting in a >10% increase in the total tube length and in a >20% increase total number of junctions, compared to control. In a wound closure assay, both H19-EVs and vector-EVs elicited a strong enough response to match that of the positive control (bFGF). In this case, all treatments significantly ($p<0.05$) decreased wound area relative to untreated MAECs, regardless of the presence of H19. This study demonstrates that H19 modulates angiogenesis in cardiac endothelial cells, encouraging its exploitation in cardiac applications. Furthermore, it portrays EVs as an effective delivery agent for lncRNAs. The authors would like to acknowledge the funding from FCT PhD Studentship (SFRH/BD/119187/2016), FCT (MITP-TB/ECE/0013/2013), QREN-COMPETE funding (Project “StrokeTherapy” co-promoted by Stemlab, Rovisco Pais and Universidade de Coimbra), Programa Operacional Competividade e Internacionalização (POCI) na sua componente FEDER e pelo orçamento da Fundação para a Ciência e a Tecnologia na sua componente OE (Project “Exo-Heart”: POCI-01-0145-FEDER-029919) and European project ERAatUC (ref. 669088). We further acknowledge the funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952266.

Abstract **#1265**

EFFECTS OF TOPOGRAPHICAL CUES ON HEMOCOMPATIBILITY OF POLYVINYL ALCOHOL HYDROGELS AND ENDOTHELIAL CELL RESPONSES

Yuan Yao[P][1], Evelyn Yim[1]

[1] *University of Waterloo*

Corresponding author's email: yuan.yao@uwaterloo.ca

Introduction: Topographical patterning of hydrogels has attracted broad attention due to its potential to affect cell behaviors and functions. In this study, we fabricated various topographies on polyvinyl alcohol (PVA) hydrogels to investigate the effects of topographical cues on hemocompatibility of the material and endothelial cell behaviors.
Materials and Methods: PVA hydrogels with different topographies were manufactured as described previously [1]. Topographies including micro-gratings, 2 µm pillars and 1.8 µm concave and convex lenses were studied. Fucoidan was conjugated on all PVA hydrogels to improve endothelial cell adhesion on PVA. Endothelial cells (ECs) were studied by immunofluorescence staining of F-actin, VE-cadherin, PECAM-1 and focal adhesion. Functional differences of ECs were assessed by monocyte adhesion assay and tube formation. Platelet adhesion and activation as well as thrombin generation on different topographies were tested to evaluate the effects of topographies on hemocompatibility.
Results: ECs formed monolayers on all types of patterned surfaces, and no significant differences on VE-cadherin expression were observed. Cells on all grating structures had significantly lower circularity and higher alignment compared other patterns. Monocyte adhesion on ECs and tube formation were affected by topographies. However, no significant differences were seen in thrombin generation on different topographical patterns. All surfaces had low platelet adhesion and activation, but no significant differences were observed among various topographies.
Conclusion: Topographies on hydrogel samples have an effect on EC functions, and grating structures induced more cell alignment. However, the hemocompatibility of patterned surfaces was not significantly different from PVA and ePTFE.

Keywords

Topography; Endothelial cell; Polyvinyl alcohol

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Abstract **#1266**

INFLUENCE OF 3D AGGREGATE CULTURE ON EPIGENETIC MEMORY AND PLURIPOTENCY MAINTENANCE IN HUMAN INDUCED PLURIPOTENT STEM CELLS

Naruchit Thanuthanakhun[P][1], Mee-Hae Kim[1], Masahiro Kino-oka[1]

[1] *Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*

Corresponding author's email: naruchit_thn@bio.eng.osaka-u.ac.jp

Although three-dimensional (3D) suspension culture is widely recognized as a promising approach for human induced pluripotent stem cell (hiPSC) manufacturing, an intracellular mechanism regulating cell pluripotency under this culture environment is still unclear. In this study, we investigated effect of 3D aggregate culture condition on cell behavioral and epigenetic alteration in association with the pluripotent state of hiPSCs. Compared with cells in two-dimensional (2D) monolayers, cells in 3D aggregates showed differential regulation of cell adhesion and Rho/Rac proteins during growth processes. In response to 3D culture, Rho was upregulated, while Rac was constantly maintained at low level. Additionally, cells in 3D culture substantially downregulated myosin phosphorylation, a key mediator of actin cytoskeletal contractility, compared with cells in 2D culture. Quantitative measurement of global histone methylation demonstrated that cells in 3D culture earlier increased the active H3K4me3 mark and persisted the repressive H3K27me3 mark throughout culture period, moreover, cells in 3D culture noticeably presented naïve pluripotent marker expression. These findings significantly extend our understandings of epigenetic and pluripotency regulation through mechanotransduction mechanism that might be taken into consideration in the design and improvement of culture environments for stem cell production.

Keywords

Human induced pluripotent stem cells; 3D culture; Epigenetic modifications

Abstract **#1267**

DUAL SCALE POROUS SCAFFOLDS BY MELT EXTRUSION

Phong Tran[P][1]

[1] *Queensland University of Technology*

Corresponding author's email: phong.tran@qut.edu.au

We combined 3D printing with porogen leaching to develop scaffolds with dual macro-micro scale porosity. By extruding a mixture of molten polycaprolactone polymer and microscale porogen particles, scaffolds with pre-determined macro-architecture were prepared, that were then leached to reveal microscale porous networks. The scaffolds had interconnected macroscale porosity of about 60% with an average pore size of 700 µm and intra-strut microscale pores with a porosity of nearly 40% and average pore size of 20–70 µm. We showed that the microscale porosity resulted in higher scaffold surface area, increased protein adsorption, faster degradation and enhanced blood clotting in vitro. We also demonstrated the use of this new type of scaffolds in regenerating bone in a rat calvarial defect and in local drug delivery for infection or cancer treatment.

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Abstract **#1268**

EXAMINATION OF VISUALIZATION OF CELL DISPERSION

Koki Abe[P][1], Takashi Kurihara[2], Shogo Miyata[3]

[1] *Shimizu Corporation Institute of Technology*

[2] *Shimizu Corporation Institute of Technology*

[3] *Keio University*

Corresponding author's email: abe_k@shimz.co.jp

Uniformed distribution of the seeded cells on to the culture surface at seeding cells are quite important for cell proliferation. Therefore, the important culture process is currently carried out by skilled workers. In order for regenerative medicine to develop in the future, mechanization of cell culture is indispensable, but there are many details are remain uncleared for developing automated cell culture systems. Therefore, we focused on cell seeding and conducted experiments on the effects of ejection shape and shaking on cell dispersion. In this report, we created a new giant liposome to visualize the dispersed state, and examined the visualization of the cell dispersion at cell seeding. We will present the results of the experiment.

Keywords

Giant Liposome; Cell dispersion

Abstract **#1269**

OXYGEN NANOBUBBLES FOR THE DELIVERY OF THERAPEUTICS AND THE REVERSAL OF HYPOXIA

Jonghoon Choi[P][1]

[1] *Chung-Ang University*

Corresponding author's email: nanomed@cau.ac.kr

Hypoxia is a key concern during the treatment of tumors, and hypoxia-inducible factor 1 alpha (HIF-1α) has been associated with increased tumor resistance to therapeutic modalities. In this study, doxorubicin-loaded oxygen nanobubbles (Dox/ONBs) were synthesized, and the effectiveness of drug delivery to MDA-MB-231 breast cancer and HeLa cells was evaluated. Dox/ONBs were characterized using optical and fluorescence microscopy, and size measurements were performed through nanoparticle tracking analysis (NTA). The working mechanism of Dox was evaluated using reactive oxygen species (ROS) assays, and cellular penetration was assessed with confocal microscopy. Hypoxic conditions were established to assess the effect of Dox/ONBs under hypoxic conditions compared with normoxic conditions. Our results indicate that Dox/ONBs are effective for drug delivery, enhancing oxygen levels, and ROS generation in tumor-derived cell lines.

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Abstract **#1270**

VASCULAR NETWORK FORMATION WITHIN 3D BIOPRINTED HEART TISSUES USING VASCULARIZED CARDIAC SPHEROIDS AS BUILDING BLOCKS

Carmine Gentile[P][1,2,3], Liudmila Polonchuk[4], Christopher D Roche[2,1], Poonam Sharma[1,2,5]

[1] *University of Technology Sydney*

[2] *University of Sydney*

[3] *Harvard Medical School*

[4] *Hoffmann-LaRoche*

[5] *University of Newcastle*

Corresponding author's email: Carmine.Gentile@uts.edu.au

The generation of a hierarchical and functional vascular network is one of the major challenges in cardiac tissue engineering. We investigated how to 3D bioprint vascularized human heart tissues by using vascularized cardiac spheroids (VCSs) in permissive hydrogels. First, VCSs were generated by co-culturing human cardiac myocytes, fibroblasts and endothelial cells in hanging drop cultures. Then, 3D bioprinted heart tissues (3DHTs) were generated by extruding VCSs mixed in alginate/gelatin-based hydrogels with optimal printability and durability. Vascular endothelial growth factor (VEGF) was applied to 3DHTs to further promote vascular network formation. Our study showed that bioprinted VCSs are viable for at least 30 days after printing and synchronously contract when electrically paced. Paired VCSs within 3DHTs fuse and generate tissues with defined geometries. VEGF promoted both angiogenic branching from VCSs into the surrounding hydrogel and spheroid fusion. Our 3D rendering analysis of 3DHTs stained with CD31 antibodies showed a branched lumenized vascular network. Altogether, our findings indicate that VCSs can be used as building blocks to 3D bioprint vascularized human heart tissues. Given their unique features in better recapitulating the vasculature typical of the human heart microenvironment, 3DHTs have the potential to be used to both prevent and treat cardiovascular disease.

Abstract **#1272**

ELUCIDATING ROLE OF SILK-GELATIN BIOINK AND TGF-BETA 3 ON THE SECRETOME OF MESENCHYMAL STROMAL CELLS IN 3D BIOPRINTED CARTILAGE CONSTRUCTS

Sourabh Ghosh[P1], Shikha Chawla[1], Giovanna Desando[2], Elena Gabusi[2], Aarushi Sharma[1], Diego Trucco[2], Cristina Manferdini[3], Mauro Petretta[2], Gina Lisignoli[2,3]

[1] *Regenerative Engineering Laboratory, Department of Textile Technology, Indian Institute of Technology, New Delhi 110016, India*

[2] *IRCCS Istituto Ortopedico Rizzoli, Laboratorio RAMSES, Bologna 40136, Italy*

[3] *Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, IRCCS Istituto Ortopedico Rizzoli, Bologna 40136, Italy*

Corresponding author's email: sourabh.ghosh@textile.iitd.ac.in

3D bioprinting attempts to fabricate engineered cartilage simulating the architectural and biological characteristics of the native cartilage. Main constraint of current approach is limited understanding of the role of bioink on molecular signaling pathways during in vitro chondrogenesis. In depth study was conducted to assess cartilage-specific molecular signaling pathways in human mesenchymal stromal cells (hMSCs) laden 3D bioprinted silk-gelatin (SF-G) constructs. Detailed proteomics analyses performed by us helped to deduce the modulation of the anabolic and catabolic chondrogenic pathways by hMSCs encapsulated in 3D bioprinted SF-G constructs. The interaction between SF-G bioink and hMSCs contributed to expression of in vivo like chondrogenic signaling pathways: Wnt/β-catenin, Wnt/PCP, HIF-1, and Notch since day 1 of chondrogenic differentiation. Additionally, we tried to elucidate the contentious role of TGF-β signaling in vitro, by evaluating the differential protein expression by hMSCs laden bioprinted constructs cultured in the presence and absence of TGF-β3. Our results demonstrated the activation of TGF-β signaling in the presence of TGF-β3 indicating a direct role of TGF-β3 addition to induce stable chondrogenic differentiation. This first-time detailed proteomics analysis would open up new avenues to identify articular cartilage-specific signaling pathways in a 3D bioprinted tissue-engineered construct to foster cartilage repair.

Abstract **#1274**

NOVEL BICISTRONIC PLASMID FOR SIMULTANEOUS GENE DELIVERY OF HGF AND VEGF165: DEVELOPMENT AND EFFICACY TESTING IN A MODEL OF LIMB ISCHEMIA

Ekaterina Slobodkina[P][1,2], Maria Boldyreva[3], Maxim Karagyaur[1,2], Vadim Balabanyan[1], Zhanna Akopyan[1,2], Yelena Parfyonova[1,3], Vsevolod Tkachuk[1,2,3], Pavel Makarevich[1,2]

[1] *Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia*

[2] *Institute for Regenerative Medicine, Medical Research and Education Centre, Lomonosov Moscow State University, Moscow, Russia*

[3] *National Medical Research Center of Cardiology Russian Ministry of Health, Moscow, Russia*

Corresponding author's email: kslobodkina@gmail.com

In this study we tested different approaches for co-expression of HGF and VEGF165 genes with known synergic effects on blood vessels formation and growth. Combined approaches by mixing 2 plasmid DNAs have limited prospect in clinical translation, so the development of a single vector carrying 2 therapeutic genes is a potential decision. We tested vectors with different design, including plasmids with internal ribosome entry sites (IRES), with bidirectional promoter or two independent promoters for each gene of interest. Studies on HEK293T and HUVEC cells showed that all plasmids provide synthesis of HGF and VEGF165 proteins with proper biological activity. However, tests in skeletal muscle explants showed dramatic difference and most plasmids failed to express HGF or VEGF165 in significant quantity. Only a bicistronic plasmid with two independent promoters provided expression of both grow factors in significant amount and near equimolar ratio. Efficacy tests of bicistronic pHGF/VEGF plasmid were performed in mouse model of hind limb ischemia. Intramuscular pHGF/VEGF administration induced significant restoration of blood perfusion compared to empty vector or saline administration which was confirmed by the difference in CD31+ capillaries density on histological sections of m. tibialis anterior.

Our study reports a first-in-class candidate gene therapy drug ready for preclinical use with shown pharmacological effectiveness in a relevant model. We assume that this construction can become a new generation gene therapy drug for "therapeutic angiogenesis" and treatment of ischemia associated diseases.

The study was supported by the state assignment of Lomonosov Moscow State University and RFBR grant #20-015-00181.

Abstract **#1275**

OSTEOCHONDRAL PELLET FORMATION USING SEQUENTIAL CENTRIFUGATION DEPOSITION.

Xuan Hao Tan$_P$[1], James, Cho Hong Goh[2]

[1] *NUS Graduate School of Integrative Sciences and Engineering*

[2] *National University of Singapore*

Corresponding author's email: e0045173@u.nus.edu

Osteochondral lesions are one of the most debilitating healthcare conditions in the world, in that it significantly affects one's quality of life. One of the promising methods to treat such lesions is to use microfracture, in which the subchondral bone is deliberately drilled in a controlled manner to allow the bone marrow to seep towards the defect site. Unfortunately, microfracture treatment typically leads to fibrocartilage formation instead of the hyaline cartilage found in native articulating cartilage tissue. This is due to the inability of the method to re-create the native structure found in articular cartilage.

This study hypothesize that the recreation of native tissue can be done if the right cell types are placed in accordance to where it needs to be found. This is performed by depositing different primary cells into a sterile tube sequentially by means of centrifugation, thus spatially controlling the co-culture of different cell types. This is performed by centrifuging a solution containing porcine nasal chondrocytes (pNCs) down first, forming a pellet. Without disrupting this initial pellet, a second solution containing porcine calvaria osteoblasts (pCOs) was centrifuged down, thereby effectively creating a pellet layer on top of the initial layer. This co-culture pellet was then cultured for up to 14 days, upon which immunohistological testing was performed. By using a double stain kit, with Collagen I and II being the targets, it was found that the pellets could form matrix of their respective regions.

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Abstract **#1276**

PREDICTING MECHANICAL ENVIRONMENT WITHIN FIBRE-REINFORCED SCAFFOLDS USING MATHEMATICAL MODELLING

Mike Chen$_P$[1], Miguel Castilho[2]

[1] *University of Adelaide*

[2] *UMC Utrecht*

Corresponding author's email: michael.chen@adelaide.edu.au

A recent approach to engineer artificial cartilage involves seeding cells within a scaffold consisting of an interconnected 3D-printed lattice of polymer fibres combined with a cast or printed hydrogel, and subjecting the construct (cell-seeded scaffold) to an applied load in a bioreactor [1]. A key question is to understand how the applied load is distributed throughout the construct.

To address this, we use mathematical modelling to describe effective properties of this elastic–poroelastic composite material [2]. We treat the fibres as a linear elastic material and the hydrogel as a poroelastic material, and exploit the disparate length scales (small inter-fibre spacing compared with construct dimensions) to derive macroscale equations governing the response of the composite to an applied load.

To validate the model, predictions from finite element simulations of the macroscale, homogenised equations are compared to experimental data describing the unconfined compression of the fibre-reinforced hydrogels. The model is used to derive the bulk mechanical properties of a cylindrical construct of the composite material for a range of fibre spacings and to determine the local mechanical environment experienced by cells embedded within the construct.

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Abstract **#1277**

A CONSOLIDATED APPROACH TO FABRICATE TUBULAR ANATOMY USING 3D PRINTING AND BIOPRINTING TECHNIQUES

Sriya YeleswarapuP[1], Shibu Chameettachal[2], Falguni Pati[2]

[1] *Indian Institute of technology, Hyderabad*

[2] *Indian Institute of Technology, Hyderabad*

Corresponding author's email: bm16m18p000001@iith.ac.in

Advances in tissue engineering of human tissues using 3D printing technology are of growing interest in developing micro physiological systems for biomedical applications. Proper designing is of important criteria while incorporating physiological and anatomical features for printing of tissues. Herein we describe the application of 3D printing technology in building tissue analogues by designing the anatomical and physiological features of tissue. We develop a simple yet effective approach using both 3D printing and 3D bioprinting technologies to construct tubular tissue constructs. In this proof of concept study, to mimic the structural similarity of bronchi, we have fabricated two concentric cylindrical structures using SLA 3D printer which are of 1 cm in height. The space in between is filled with decellularized smooth muscle extracellular matrix (dSMM) bioink. The surfaces of the cylinders have holes to enable efficient perfusion of media to the tissue. This structure provides support to the mechanically weak bioink to retain its shape while printing. The structure was cultured for 21 days under dynamic conditions in an in-house printed bioreactor. Characterization assays revealed viable cells that are functional in dSMM within the structure. We believe that this method can be applied to various cylindrical structures of human scale. Tissues such developed can be used for drug testing or for understanding many biological mechanisms or as a direct tissue-engineered implant for in vivo applications. We anticipate that this strategy will help us to overcome one of the key challenges of mechanical instability of decellularized ECM based bioinks during printing.

Abstract **#1278**

PERIVASCULAR NICHE-ON-A-CHIP FOR INTRACELLULAR LABEL-FREE INVESTIGATION OF MESENCHYMAL STEM CELLS METABOLISM

Simone Perottoni[1], Nuno Neto[2], Cesare Di Nitto[1], Michael Monaghan[2], Manuela Teresa Raimondi[1]

[1] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy*

[2] *Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland*

Corresponding author's email: simone.perottoni@polimi.it

Mesenchymal stem cells (MSCs) experience many intercellular and extracellular forces and specific oxygen tension (pO2) inside their native in vivo stem cell perivascular niche. Many questions still remain regarding the complexity of this micro-environment and how oxygen concentration gradients and shear forces at the niche-tissue interface can affect MSC metabolism; which recently showed to play a determinant role in stemness maintenance or differentiation. Here by the use of a microbioreactor (MOAB) [1] suitable for high-resolution imaging coupled with fluorescence lifetime imaging microscopy (FLIM) [2] we profile cellular metabolism of human-MSCs (h-MSCs) in a simulated stem cell niche environment by controlling shear stress and pO2 gradients via interstitial flow velocity levels. The combination of these advanced modalities can reveal metabolic activity of cells in very specific micro-anatomic environments to analyse physiology and diseases. We were able to finely tune and detect the pO_2 spatial depletion along the micro-chamber in a range of interstitial flow velocities mimicking the transvascular extravasation of fluids during changings in vessels permeability. According to the specific decay in oxygen cells uptake, we detected a significant spatial metabolic transition towards glycolytic activity by measuring a bound NADH fraction linear decay towards the outlet hypoxic region. Our microfluidics/imaging platform demonstrated to sustain a microphysiological environment for stem cells culture enabling real-time high-resolution imaging of metabolic activity representing a powerful tool for MSC studies in metabolic controlled conditions.

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Abstract **#1279**

DEVELOPMENT OF A PATIENT-DERIVED OSTEOBLAST/OSTEOCLAST 3D IN VITRO MODEL FOR THE STUDY OF BREAST CANCER BONE METASTASES

Valerie Nitschke[1,2], Brittany Sanker[1,3], Nathan Pavlos[4], Nathalie Bock[1]

[1] *Queensland University of Technology*

[2] *Technical University of Munich - School of Medicine*

[3] *University of Queensland*

[4] *University of Western Australia*

Corresponding author's email: valerie.nitschke@web.de

Bone metastases are a common complication of highly prevalent cancer types such as breast and prostate cancers. They result in imbalances between bone-forming osteoblasts and bone-degrading osteoclasts and affect bone density, resulting in painful lesions. Yet, few in vitro models encapsulate both bone cell types to simultaneously study such interactions. Here we developed a patient-derived co-culture 3D model to study the reciprocal effects of osteoblasts/osteoclasts and a metastatic breast cancer cell line MDA-MB-231.Osteoblasts derived from human bone (hOBs) were cultured on 3D printed calcium phosphate-coated medical-grade polycaprolactone scaffolds and grown to obtain a highly mineralized matrix [1]. In parallel, human peripheral blood monocytes were differentiated into mature osteoclasts (hOCs), as shown by colorimetric assays, immunofluorescence (TRAP, CatK) and resorption pit assessment. The mature hOCs were then seeded on top of the hOB-seeded mineralized scaffolds and co-culture morphology and mineralisation (microCT) were further characterized.We found that the addition of MDA-MB-231 cells seeded on top of the constructs resulted in increased cancer cell proliferation when hOCs were present. As expected with breast cancer metastases, a decrease in mineralisation was also observed in the hOC/hOB-scaffolds after cancer cell seeding and co-culture for 3 weeks, consistent with osteolytic metastases.Including two of the key cellular drivers of bone remodeling in a reproducible tissue-engineered model therefore provides an advanced and broadly applicable research and drug testing platform of cancer metastases in bone.

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Abstract **#1280**

INDUCING HUMAN RETINAL PIGMENT EPITHELIUM-LIKE CELLS FROM SOMATIC TISSUE

Cody Kime[P][1], Ivo Ngundu Woogeng[P][1]

[1] *RIKEN Center for Biosystems Dynamics Research*

Corresponding author's email: ivo.woogeng@riken.jp

Regenerative medicine relies on basic research to find safe and useful outcomes that are only practical when cost-effective. The human eyeball requires the retinal pigment epithelium (RPE) for support and maintenance that interfaces the neural retina and the choroid at large. Nearly 200 million people suffer from age-related macular degeneration (AMD), a blinding multifactor genetic disease among other retinal pathologies related to RPE degradation. Recently, autologous pluripotent stem cell-derived RPE cells were prohibitively expensive due to time, therefore we developed a new simplified cell reprogramming system. We stably induced RPE-like cells (iRPE) from human fibroblasts by conditional overexpression of broad plasticity and lineage-specific pioneering transcription factors. iRPE cells showed features of modern RPE benchmarks and significant in-vivo integration in transplanted chimeric hosts. Herein, we detail the iRPE system with comprehensive modern single-cell RNA (scRNA) sequencing profiling to interpret and characterize its best cells. We anticipate that our system may enable robust retinal cell induction for regenerative medicine research and affordable autologous human RPE tissue for cell therapy.

Keywords

Cell Reprogramming; Retinal Pigment Epithelium; Biomedicine

Abstract **#1281**

TAT KAPPA (TATK): A NOVEL CELL PENETRATING PEPTIDE FOR DELIVERY OF PLURIPOTENT PROTEINS INTO TARGET CELLS

Fazlina Nordin$_P$[1], Ubashini Vijakumaran[1], Zariyantey Abdul Hamid[1], Maha Abdullah[2], Gee Jun Tye[3]

[1] *Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur, Malaysia*

[2] *University Putra Malaysia (UPM), Selangor, Malaysia;*

[3] *Universiti Sains Malaysia (USM), Penang, Malaysia*

Corresponding author's email: nordinf@ppukm.ukm.edu.my

Induced Pluripotent Stem Cell (iPSC) holds a magnificent place in the medical revolution. Since Yamanaka's iPSC discovery in 2006, several works of research have proven that the enforced expression of transcription factors Oct-3/4, KLF4, and Sox2 in differentiated and matured cells induce genetic reprogramming of the cells into iPSC. However, the conventional reprogramming method of using viral vectors confers greater risk for unwanted genetic modification. Therefore, this current study utilised a non-viral reprogramming approach of using a novel protein transduction domain, namely trans-activator of transcription kappa (TATκ), a synthetic TAT-HIV, to deliver these transcription factors genes as an alternative method for iPSC generation. With this new strategy, this study established a stable clone of 293T cells expressing and secreting TATκ fusion pluripotent proteins (TATκ-KLF4, TATκ-Sox2, and TATκ-Oct-3/4). TATκ-GFP, a marker protein, and pluripotent proteins transduced the targeted (U937) monocyte cell line, proved this TATκ possesses the ability to penetrate across the cell membrane. Post 20 days of transduction, U937 cells elongated and started to adhere. However, the presence of bona fide iPSC colonies was unable to be obtained, which might have been due to the incomplete reprogramming or insufficient duration of protein transduction in generating iPSC cells.

Keywords

Cell-penetrating peptide; Transduction; Induced Pluripotent Stem Cells

Abstract **#1282**

FABRICATION OF 3D BIO-PRINTED NANOCELLULOSE/ALGINATE HYDROGEL SCAFFOLDS FOR DELIVERY OF PANCREATIC ISLETS

Shadab Abadpourp[1,2,3], Essi M Niemi[2,3,4], Yadong Zhao[5], Eric Thompson[5], Christofer Troedsson[5], Paul Gatenholm[6], Hanne Scholz[1,2,3]

[1] *Department of Transplant Medicine, Oslo University Hospital, Oslo, Norway*

[2] *Institute for Surgical Research, Oslo University Hospital, Oslo, Norway*

[3] *Hybrid Technology Hub, Centre of Excellence, Institute of Basic Medical Sciences, University of Oslo, Norway*

[4] *Department of Vascular Surgery, Oslo University Hospital, Oslo, Norway*

[5] *Ocean TuniCell, Blomsterdalen, Norway*

[6] *CELLHEAL AS, Norway*

Corresponding author's email: shadab.abadpour@rr-research.no

Islet transplantation is a minimally invasive beta cell replacement therapy with the potential to cure Type 1 Diabetes (T1D). However, success rates are hampered by loss of islet transplanted in portal veins. Developing scaffolds that favor islet survival and allow transplantation in extra-hepatic sites is one solution to achieve a cure for T1D. Indeed, 3D-bioprinting has shown good islet viability and morphology. Delivered with biocompatible hydrogels, this approach could favor islet survival and reduce cellular stress.

Here, we fabricated implantable scaffolds for human islets using clinical-grade nanocellulose (80%)/alginate (20%) hydrogel (TUNICELL from Ocean TuniCell). Islets were embedded in hydrogel scaffolds using the INKREDIBLE+ 3D-bioprinter (CELLINK, Sweden). Fluorescent Recovery after Photo-bleaching with 3-5 kDa and 70 kDa dextrans was used to evaluate the diffusion properties of the hydrogel scaffolds. Cell viability and functional physiology were assessed using FDA/PI staining and glucose-stimulated insulin secretion on days 1, 8 and 13 post-print.

Our hydrogel showed 100% recoveries after photo-bleaching in 4-6 sec, or 5-18 sec, for scaffolds incubated with 3-5 kDa and 70 kDa dextrans, respectively. In vitro assessments of bio-printed scaffolds revealed viable islets on days 1, 8 and 13 post-print. Islets in scaffolds showed proper insulin secretion in response to basal and stimulated glucose levels over time (e.g. at day 8: basal insulin 481.3 ± 40.9, stimulated insulin 1186.0 ± 6.2, post-stimulation insulin 973.0 ± 12.2, n=4).

This study indicates that our hydrogel has proper diffusion properties that create a favorable islet micro-environment resulting in preserved viability and physiology of islets.

Abstract **#1283**

FORMULATION AND APPLICATION OF SERICIN BASED HYDROGEL AS A PHOTOINITIATOR FREE BIOINK

Jeong Ok Lim[P][1,2], Jeung Soo Huh[3,4], Mijin Jang[5,6]

[1] *Kyungpook Naitonal University Hospital*

[2] *Kyungpook National University, School of Medicine*

[3] *School of Materials Science and Engineering*

[4] *Kyungpook National University*

[5] *Laboratory Animal Center*

[6] *Daegu Gyeongbuk Medical Innovation Foundation*

Corresponding author's email: jolim@knu.ac.kr

Background: Ideal printable biomaterials are demanding as tissue printing fields are explored. However, development of proper materials having good printability and photo-initiator free is challenging. Commonly used cross linking agents such as methanol, glutaraldehyde, EDC-NHS, photo-initiator and exposure to UV light would not be suitable for biocompatibility
Objectives: We investigated the possibility of using silk sericin combined with gelatin and glycerol to obtain this ideal 3D printable self-gelling biomaterial and evaluated its application potential for wound dressing patch in vitro and in vivo.
Methods: Formulation of sericin based hydrogels, and its physico-chemical property associated with printability, and biological properties in vitro and in vivo were investigated.
Results: Empirical data demonstrated the safety and effectiveness of sericin-gelatin-glycerol complex as a printable hydrogel. Silk sericin aqueous solution has a characteristic of forming a gel as a structural change from a random coil to a beta sheet occurs. Gelatin has high water absorption capacity and non-immunogenicity and biodegradability. Gelatin is a solid at low temperatures and has poor mechanical properties. Sericin was mixed with gelatin rendering stability under physiological conditions. Richness of acidic amino acids in sericin and basic amino acids in gelatin can be utilized for stable bonding which gives structural integrity of the complex. Another component in the complex, glycerol acts as plasticizer that gives flexibility to sericin.
Conclusion: A novel sericin-hydrogel produced a photoinitiator-free bioink. In vitro and in vivo studies demonstrated the safety and effectiveness of sericin-hydrogel as a printable patch for wound healing dressing.

Keywords

Bioink; Photoinitiator-free; sericin

Abstract **#1284**

MICROTECHNOLOGIES AS ENABLING TOOLS FOR THE DEVELOPMENT OF ADVANCED ORGANS-ON-CHIP MODELS

Marco Rasponi[P][1], Roberta Visone[1], Erika Ferrari[1], Paola Occhetta[1]

[1] *Politecnico di Milano*

Corresponding author's email: marco.rasponi@polimi.it

Organs-on-chip (OoC) refer to microfluidic in vitro models recapitulating functions of human organs. OoC provide unprecedented opportunities to recreate tissue-specific microenvironments and to analyze functional readouts. We here present a series of innovative technologies specifically designed to enhance different OoC features (i.e. integration of mechanical stimulation, 3D interfaces and sensors).

The uBeat® technology allows to provide three-dimensional cell microconstructs with a controlled mechanical stimulation, which can be tuned to model different patho-physiological states and tissues. Human cardiomyocytes derived from induced pluripotent stem cells were subjected to a uniaxial strain, yielding to functional cardiac microtissues in a beating heart-on-chip model[1]. The same stimulation applied to co-cultures of cardiomyocytes and cardiac fibroblast generated either fibrotic or pro-fibrotic states[2]. A pure confined compression was instead applied to a human cartilage-on-chip model, demonstrating the possibility to trigger an osteoarthritic phenotype merely with mechanical overload[3].

A technology to obtain multicompartmental microtissues was developed to generate neat interfaces between different 3D cell microconstructs, achieving both continuous (osteochondral) and barrier (blood-brain barrier, BBB) models[4].

Blind-ended microfluidic channels were implemented as guides for the precise insertion of microelectrodes in OoC models. Applied to the beating heart-on-chip, this technology allowed the continuous recording of cardiac electrophysiological parameters[5]. Moreover, electrodes were used in a BBB model to assess barrier functionality via trans endothelial-epithelial resistance measurements[6].

Integration and combination of described technologies in OoC setups thus allows not only to faithfully recapitulate the physical native cell microenvironment, but also to improve their applicability in biological studies, disease modelling and drug screening.

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Abstract **#1285**

FRONT-LINE APPLICATION OF NEGATIVE PRESSURE WOUND THERAPY (NPWT) AND NON-VIRAL GENE AUGMENTATION FOR ENHANCED HEALING OF COMBAT-ACQUIRED POLY-TRAUMA

James Dixon[P][1], Amelie Savers[1]

[1] *University of Nottingham*

Corresponding author's email: james.dixon@nottingham.ac.uk

Current combat casualty care is based on rapid evacuation, and 'golden hour' interventions aim to provide life and tissue saving therapies in austere environments. Negative-pressure wound therapy (NPWT) is extensively deployed and is under-explored as a delivery system for front-line therapeutics. Our work has exploited this unique opportunity to marry enhanced delivery of growth-factor gene therapy with the biomechanical stimulatory activities of NPWT; aiming to enhance vascularisation and inhibit negative inflammatory responses. The goal of our strategy, delivered by non-viral gene therapy, is to precondition traumatised tissue for subsequent surgery and to enhance tissue retention and healing. We are exploiting our peptide nanoparticle (NP) system (termed Glycosaminoglycan-binding enhanced transduction; GET) which can be freeze-dried, reconstituted and administered by injection. To test our technology we are exploiting ex-vivo culture of abdominal full-thickness porcine or human skin. In this model we wound skin and test the delivery and effectiveness of our systems. We have also modified clinically deployed NPWT systems to apply pressure to these samples looking for synergy. We have demonstrated reporter gene and multiple growth factor/cytokine expression, such as VEGF and PDGF to enhance vascularisation, and in combination with IL10 and IL1Ra as anti-inflammatory cytokines. The therapy can be reapplied and a single dose can generate therapeutic levels in wounds for at least 2 weeks. Our ultimate goal is that state-of-the-art genetic augmentation approaches can be exploited in austere environments, applying regenerative medicine to the front-line.

Abstract **#1286**

RAPID INTRAOPERATIVE GENETIC AUGMENTATION OF AUTOGRAFTS FOR RECONSTRUCTIVE SURGERY OF SEVERE TRAUMA

James Dixon$_P$[1], Trang Thi Mai[1], Jane McLaren[2], Benjamin Ollivere[1], Brigitte Scammell[1], Hoda Eltaher[1]

[1] *University of Nottingham*

[2] *University of Nottingham*

Corresponding author's email: james.dixon@nottingham.ac.uk

Survivors with severe tissue injury have unmet clinical needs for better bone and soft tissue regeneration. Specifically they have progressive tissue loss due to inflammation, necrosis, infection and the development of critical size bone defects is a surgical challenge. The Reamer/Irrigator/Aspirator (RIA) system is used to recover patient bone (and stem cells) for autografting in severe trauma & infections. Addition of growth-factors (GFs) can be transformative is such systems; however, their short-term activity requires expensive high doses with significant side effects. We have developed a simple system to express any therapeutic gene in cells and tissues, including GF genes. Our GET technology acts 'transiently' meaning therapeutic proteins such as GFs are produced directly by the 'transfected' tissue over a short therapeutic period. We have previously shown that GF gene therapy (BMP2/VEGF) in rat calvarial defects can enhance bone repair both safely and cheaply (Raftery et al. 2019). Here we have exploited the reserve of stem cells in bone marrow (BM) and grafts, and the GET system to augment stem cell activity by rapid genetic augmentation of BMP2 and VEGF genes with the aim to re-administer them to augment reconstructive surgery. We demonstrate the efficacy of the gene delivery approach and the enhancement of ex vivo osteogenesis by graft-expressed BMP2 and VEGF augmentation. By delivering genes that cause the resident cells at the defect site to produce regulated levels of GFs could be the transformative approach needed to allow safe and effective bone regeneration for treating many diseases or injuries.

Abstract **#1287**

RAPIDLY TRANSDUCING AND SPATIALLY LOCALIZED MAGNETOFECTION USING PEPTIDE-MEDIATED NON-VIRAL GENE DELIVERY

James Dixon[P][1], Lia Blokpoel Ferreras[1], Sze Yan Chan[2], Saul Vazquez Reina[2]

[1] *University of Nottingham*

[2] *University of nottingham*

Corresponding author's email: james.dixon@nottingham.ac.uk

Non-viral delivery systems are generally of low efficiency, which limit their use in gene therapy and editing applications. We previously developed a technology termed glycosaminoglycan (GAG)-binding enhanced transduction (GET) to efficiently deliver a variety of cargoes intracellularly; our system employs GAG-binding peptides which promote cell targeting, and cell penetrating peptides (CPPs) which enhance endocytotic cell internalization. Herein, we describe a further modification by combining gene delivery and magnetic targeting with the GET technology. We associated GET peptides, plasmid (p)DNA and iron oxide superparamagnetic nanoparticles (MNPs); allowing rapid and targeted application of GET-mediated uptake by application of static magnetic fields. This produced effective transfection levels with seconds to minutes of exposure, and localized gene delivery two orders of magnitude higher in targeted over non-targeted cell monolayers using magnetic fields. More importantly, high cell membrane targeting by FLR-DNA-MNP co-complexes and magnetic fields allowed further enhancement to endocytotic uptake, meaning that the nucleic acid cargo was rapidly internalized beyond that of GET complexes alone (FLR-DNA). Magnetofection combined with GET-mediated delivery allows magnetic field-guided local transfection in vitro, and could facilitate focused gene delivery for future regenerative and disease-targeted therapies in vivo.

Abstract **#1288**

STROMAL LENTICULE BANKING FOR FUTURE CORNEAL REFRACTIVE MODIFICATIONS: PROOF-OF-CONCEPT STUDY

Andri K Riau$_P$[1,2], Kenny Boey[3], Gwendoline Goh[1], Nur Zahirah binte Yusoff[1], Huiying Tan[3], Kin Fai Tang[3], Gary H F Yam[4], Jodhbir S Mehta[1,2,5]

[1] *Tissue Engineering and Cell Therapy, Singapore Eye Research Institute, Singapore*

[2] *Eye-Academic Clinical Program, Duke-NUS Medical School, Singapore*

[3] *Group Laboratory Operation, Cordlife Group Limited, Singapore*

[4] *Department of Ophthalmology, University of Pittsburgh, USA*

[5] *Singapore National Eye Center, Singapore*

Corresponding author's email: andri_riau@yahoo.com

Since 2010, over 2 million small incision lenticule extraction (SMILE) procedures have been performed. The number will gradually supersede the LASIK due to the flap-less nature and faster visual recovery. The byproduct of the procedure is a refractive lenticule, which can be reimplanted within the stroma to correct refractive errors (e.g., presbyopia) and restore corneal volume of keratoconus patients [1,2]. Nevertheless, standardization of cryopreservation and regulatory adherence has to be followed to improve the practicality and worldwide implementation of lenticule banking. Here, we showed a proof-of-concept of lenticule banking service, established by Cordlife in collaboration with our group. Human lenticules, collected from our clinic, were stored for 1 year in DMEM+10% DMSO cryoprotectant in vapor-phase liquid nitrogen in Cordlife's cryofacility. After cryopreservation, the tissue transparency appeared reduced from 95% (fresh) to 90% due to the reduction in collagen fibrillar distance from 63.5±5.4 nm to 50.3±4.6 nm ($p<0.001$). The central 3mm of the thawed lenticules was trephined and implanted in 3 rabbits' stromal pockets for 4 months (steroids and antibiotics were withdrawn after 2 months). On the slit-lamp and in vivo confocal microscope, the lenticules appeared hazy for the first 4 weeks but resolved subsequently. The surrounding tissue, however, was clear. The corneal epithelial, stromal, and endothelial cells were unaffected by the implantation. Immunohistochemistry of the corneas revealed no fibrosis (fibronectin, CD90, and α-smooth muscle actin negative) but mild tissue reparation (tenascin-C and COL3A1) at the lenticular edge. In summary, we have demonstrated the feasibility and safety of long-term lenticule banking.

Keywords

tissue banking; presbyopia; in vivo

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Abstract **#1289**

IMAGING OF METABOLIC ACTIVITY ADAPTATIONS TO UV STRESS, DRUGS AND DIFFERENTIATION AT CELLULAR RESOLUTION IN SKIN AND ORGANOTYPIC SKIN EQUIVALENTS

Florian Gruber[P][1,2], Christopher Kremslehner[1,2], Anne Miller[1], Robert Nica[3], Ionela-Mariana Nagelreiter[1], Bahar Golabi[1], Michael Mildner[1], Erwin Tschachler[1], Francesca Ferrara[4], Kristaps Klavins[5], Markus Schosserer[6], Johannes Grillari[7,2], Arvand Haschemi[1]

[1] *Medical University of Vienna*

[2] *Christian Doppler Laboratory for the Biotechnology of Skin aging*

[3] *TissueGnostics GmbH*

[4] *North Carolina State University*

[5] *CeMM Research Centre for Molecular Medicine*

[6] *University of Natural Resources and Life Sciences*

[7] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology*

Corresponding author's email: florian.gruber@muv.ac.at

The epidermis is a multi-layered epithelium that consists mainly of keratinocytes which proliferate in its basal layer and then differentiate to form the stratum corneum. During differentiation keratinocyte composition, function and metabolism changee. Stressors, like ultraviolet (UV) radiation thus differently affect epidermal keratinocytes, depending on differentiation stage. Exposure to UV elicits DNA damage responses, detoxification, repair or death. Rapid diversion of glucose flux into the pentose phosphate pathway (PPP) is a mechanism to rapidly generate reduction equivalents and nucleotide precursors – after UV damage. There is little known about the correlation of such metabolic activity with differentiation state, cell damage and tissue localization of epidermal cells. We developed a method to correlate the activity of glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme of this metabolic UV response, at cellular resolution to cell type, differentiation state and cell damage in human skin and in organotypic skin equivalents (SE). We thereby could verify rapid activation of G6PD as an immediate UVB response not only in basal but also in differentiating epidermal keratinocytes and found increased activity in cells which initiated DNA damage responses. UVB treatment of keratinocytes before organotypic culture led to abnormal distribution in the SE and reduced G6PD activity compared to neighboring cells. Finally, we found that the anti-diabetic and potential anti-aging drug metformin strongly induced G6PD activity throughout the SE. PPP activation may be useful to enhance the skin's antioxidant defense systems and DNA damage repair capacity on demand.

Keywords

Metabolism; Multimodal Imaging; Aging

Abstract **#1290**

ADVANCED HOLLOW CATHODE DISCHARGE PLASMA TREATMENT OF NOVEL BI-LAYERED FIBROUS GUIDANCE CONDUITS FOR ENHANCED PERIPHERAL NERVE REGENERATION

Sheida Aliakbarshirazi[P][1], Mahtab Asadian[1], Rouba Ghobeira[1], Mehrnoush Narimisa[1], Iuliia Onyshchenko[1], Anton Nikiforov[1], Heidi Declercq[2,3], Nathalie De Geyter[1], Rino Morent[1]

[1] Research Unit Plasma Technology (RUPT), Department of Applied Physics, Faculty of Architecture and Engineering, Ghent University, Belgium

[2] Tissue Engineering Lab, Department of Development and Regeneration, Faculty of Medicine, KU Leuven Campus Kulak, Belgium

[3] Tissue Engineering Group, Department of Human Structure and Repair, Faculty of Medicine and Health Sciences, Ghent University, Belgium

Corresponding author's email: sheida.aliakbarshirazi@ugent.be

Nowadays, peripheral nerve injuries leading to acute disabilities still constitute a major clinical burden. Typical surgical procedures such as direct repair or autografting approaches are often limited by the nerve gap size and/or by several drawbacks. As an alternative, nerve guidance conduits (NGCs) comprised of biodegradable polymers have been tissue-engineered to guide axons and enhance nerve regeneration. Nonetheless, most of the developed NGCs do not concurrently possess both topographical and biochemical cues that recapitulate the properties of the extracellular matrix. Thus, good results in treating only small defects were achieved. Therefore, in order to repair critical nerve gaps, NGCs with advanced physicochemical properties are aimed at. To do so, electrospinning is employed to generate an NGC made of polycaprolactone/polylactic-co-glycolic acid with outer and inner walls composed of random and highly aligned nanofibers respectively. This design guiding axon growth (aligned fibers) while permitting nutrient supply (random fibers) is obtained in a one-step process using an innovative 2-pole air gap collector. In order to reach and bioactivate the inner NGC wall without damaging the nanofibers, a novel surface plasma treatment is performed making use of a purposely designed plasma reactor consisting of a hollow cathode discharge sustained in O2. X-ray photoelectron spectroscopy results reveal a successful incorporation of oxygen-containing functionalities on the inner wall thus enhancing its hydrophilicity. An extensive in vitro study involving PC12 cells shows greatly improved cell adhesion, proliferation and extension of unidirectional long neurites on plasma-treated NGCs. Overall, the proposed NGCs demonstrate great potential in nerve regeneration.

Keywords

Electrospinning; Nerve guidance conduits; Plasma treatment

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Abstract **#1291**

DESMOSOMAL ASSEMBLY IN INTERFOLLICULAR STEM CELLS (IFSCS) VIA DESMOSOMAL AND CLASSICAL CADHERIN PROTEIN NANOPATTERNS

Sadegh Ghorbani[P][1], Ali Shahrokhtash[1], Kei Bech[1], Fiona Watt[2], Duncan Sutherland[1]

[1] *Interdisciplinary Nanoscience Center (iNANO) and CellPAT Center, Aarhus University, Aarhus C, Denmark*

[2] *Centre for Stem Cells and Regenerative Medicine, King's College London, 28th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, United Kingdom*

Corresponding author's email: sagh@inano.au.dk

Adhesion between intrafollicular stem cells (IFSCs) is established by the formation of specific disc-like (200-500 nm in diameter) cellular junctional complexes including desmosomes. The desmosomal cell-cell attachments, especially in mechanically challenged tissue such as epidermis, are composed of two types of cadherins, desmocollins (DSC) and desmogleins (DSG). Of the DSG's, DSG2 is expressed only in the basal epidermal layer. Regarding the dynamic structure of desmosomal junctions, other cell-cell adhesion may also play a role during assembly. The initial cell-cell binding through adherence junctions (via E-cadherin) may facilitate assembly of immature desmosome junctions and finally lead to the development of mature desmosomes via DSG-DSC heterobinding. However, their combinatorial roles in desmosome assembly and cell behavior are not well understood in the epidermis layer.

In this work, nanopatterns of DSG2 and Ecad are created as mimics of the cellular interface and used to study the formation of desmosomal assemblies in adherent IFSC cells. Nanoscale engineering was used to create an inorganic chemical pattern which was used to assemble oriented Dsg2 Fc or Ecad Fc into created arrays of circular nanopatterns of the Dsg 2 Fc, Ecad Fc or their combination (50:50) with lateral size of 100, 300 or 500 nm. Between the protein nanopatterns, cell adhesion studies show that IFSCs adhere to the Dsg2Fc and EcadFc patterns and start differentiation or remain undifferentiated status under different conditions. The preliminary results show that the size of nanopatterned with desmosomal and adherence cadherins separately or together alter desmosomal formation.

Keywords

Desmosome junction ; Interfollicular stem cells (IFSCs); Nanopattern

Abstract **#1292**

RESTORATION OF THE HAIR-INDUCTIVE CAPACITY OF HUMAN DERMAL PAPILLA CELLS EMBEDDED IN COLLAGEN MICROGEL

Monami Yamane$_P$[1], Tatsuto Kageyama[1,2], Junji Fukuda[1,2]

[1] *Faculty of Engineering, YOKOHAMA National University, Yokohama, Kanagawa, Japan*

[2] *Kanagawa Institute of Industrial Science and Technology (KISTEC), Japan*

Corresponding author's email: yamane-monami-sx@ynu.jp

Hair regenerative medicine has emerged as a promising approach for the treatment of hair loss. Considering the embryonic development of hair follicles is triggered by the formation of a hair follicle germ (HFG), we have proposed an in vitro preparation approach of HFGs and demonstrated efficient hair follicle generation upon transplantation into mice[1]. However, this approach is still challenging mainly because follicular stem cells (i.e., dermal papilla cells (DPCs)) tend to lose their hair-inductive capacity during subculture in traditional 2D expansion culture conditions[2]. In this study, we propose an approach to restore the hair-inductive capacity of human DPCs using collagen microgels in the HFG preparetion[3]. DPCs were encapsulated in 2-µl collagen microgels, which spontaneously contracted from 2 mm diameter to less than 700 µm during three days of culture though cell attraction forces. This phenomenon is probably similar processes observed in the earliest morphogenesis of hair follicle generation. Interestingly, this contraction was crucial for upregulating hair-induction markers including alkaline phosphatase gene of DPCs. The collagen microgels containing DPCs prepared in this approach were mixed with freshly isolated mouse embryonic epithelial cells and formed HFGs. De novo hair follicle and hair shaft generation were observed after transplantation of the HFGs into the backs of nude mice. This could be a practical approach to restore the hair-inductive capacity of DPCs for hair regenerative medicine.

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Abstract **#1293**

MELT ELECTROWRITING ENABLES FABRICATION OF BIOMIMETIC HIERARCHICAL SCAFFOLDS

Małgorzata Włodarczyk-Biegun[1,2,3], Piotr Zieliński[1], Magdalena Gładysz[1], Anno Hofman[1], Xixi Wu[1], Maria Villiou[2], Marcus Koch[2], Aranzazu del Campo[2], Merleen Kamperman[1]

[1] *University of Groningen, Groningen, The Netherlands*

[2] *Leibniz Institute for New Materials, Saarbrücken, Germany*

[3] *Biotechnology Center, Silesian University of Technology, Gliwice, Poland*

Corresponding author's email: m.k.wlodarczyk@rug.nl

Native tissues are highly organized structures, with an intricate cellular microenvironment. They are non-homogeneous and typically exhibit functional gradients in their architecture and composition over very small length-scales. Therefore, their regeneration is extremely difficult, and it is still a major challenge to produce biomimetic scaffolds using traditional manufacturing processes.

In our study, we propose to employ an emerging biofabrication technique: Melt Electrowriting (MEW), to more closely reproduce the complexity of native tissues. MEW, combining the fundamental principles of electrospinning and 3D printing, allows for precise control over material placement and high printing resolution. As a result, scaffold's morphology can be controlled at both macro and micro levels. We show that by finely tuning scaffolds design and controlling their structure, systems with mechanical and biological performance relevant for different tissues can be obtained. Particularly, we present the utility of MEW for reconstruction of the Human Trabecular Meshwork (membrane located in the eye), hard-soft interfaces and skin tissue.

We envision that the small-scale biomimetic scaffolds printed with high precision will serve in the future as implantable systems or an in-vitro more adequate testing models.

Abstract **#1294**

FABRICATION OF HALAL-BASED GELATIN SPONGE FOR FUTURE USE AS BIOFUNCTIONAL SKIN SUBSTITUTE

Mohd Heikal Mohd Yunus$_P$[1,2]

[1] *Department of Physiology, UKM Medical Centre, Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur.*

[2] *Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur.*

Corresponding author's email: mohdheikalyunus@yahoo.com

Gelatin has the intended biological properties resembling the native skin and potentially fabricated as skin substitute for full thickness wound treatment. This study aimed to fabricate and characterise the certified halal bovine gelatin using various crosslinking method. Porous gelatin sponge was fabricated by the freeze-drying approach. Crosslinking of gelatin sponge was completely performed via chemical or physical intervention by using genipin (GNP), 1-ethyl-3-(3-dimethylaminopropyl) (EDC), or dihydrothermal (DHT) method, respectively. The physicochemical, biomechanical, cellular biocompatibility and cell-biomaterial interaction with the fabricated gelatin sponge were evaluated. Increase in concentration and double croslinking, gradually increase crosslinking degree, enzymatic hydrolysis resistance, thermal stability, porosity, wettability, and mechanical strength were observed. However, the pore size, water retention, and water absorption ability were found decreasing trend. No changes in chemical content and crystal structure were observed. Human epidermal keratinocytes (HEK) and human dermal fibroblasts (HDF) revealed slight toxicity with double crosslinking. HEK and HDF attachment and proliferation remain similar with each crosslinking approaches. Immunogenicity was observed to be higher in double-crosslinking compared to the single crosslinking intervention. The selected crosslink gelatin sponge showed potential to be tailored according to a precise need of wound healing process by manipulating the crosslinking process.

Abstract **#1295**

MECHANICAL PROPERTIES OF FIBRIN-BASED HYDROGELS WITH COVALENT AND SUPRAMOLECULAR CROSSLINKS

Miriam Aischa Al Enezy-Ulbrich[P][1,2], Nicole Terefenko[1,2], Shannon Anna Jung[1,2], René Plum[1,2], Norina Labude-Weber[3,4], Hanna Malyaran[3,4], Svenja Wein[3,4], Sabine Neuss[3,4], Andrij Pich[1,2]

[1] *Institute for Technical and Macromolecular Chemistry, Research Area Functional and Interactive Polymers, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany*

[2] *DWI - Leibniz Institute for Interactive Materials, Forckenbeckstraße 50, 52074 Aachen, Germany*

[3] *Helmholtz Institute for Biomedical Engineering, BioInterface Group, RWTH Aachen University, Pauwelsstrasse 20, 52074 Aachen, Germany*

[4] *Institute of Pathology, RWTH Aachen University, Pauwelsstrasse 30, 52074 Aachen, Germany*

Corresponding author's email: miriam.al.enezy@rwth-aachen.de

In modern implant development, researchers focus on the enhancement of both biocompatibility and reproducibility. Even though cardiovascular implants are commonly used, they only have a limited lifetime which also affects their properties and functionality. Many research activities face the optimisation of implant properties. Formation of blood clots in mechanical implants (heart valves), bacterial biofilms (dental implants) or the degradation of bioprotheses should be prevented. In addition, the organ implant may still be rejected by the patient's body.

The aim of this work is the synthesis of a biohybrid heart valve consisting of a fibrin-based gel-matrix with a textile reinforcement. We could already demonstrate that the concentration of a linear poly(N-vinylpyrrolidone)-copolymer with functional epoxide groups has an influence on the mechanical behaviour of the hydrogel due to covalent crosslinking. Specific fibrin-binding peptides exhibit supramolecular interactions within the fibrin-matrix. Their influence on the mechanical properties of the fibrin-based hydrogels can be characterized by rheological measurements. The use of both, covalent and supramolecular crosslinkers, expand our innovative functional tool-box for fibrin-based biohybrid hydrogels for the patient-specific individualisation of implants. The hydrogel serves as an attractive environment for human stem cells. Experiments demonstrate that the use of copolymers have a high impact on the hydrogels' properties and their long-term stability. For the characterization various concentrations of fibrin-binding peptides and copolymer have been used. Future experiments will deal with the analysis of the cytocompatibility of the fibrin-binding peptides. Furthermore, the adhesion of the hydrogels on various substrates will be tested.

Abstract **#1297**

COMPARISON OF DIFFERENT TRANSFECTION METHODS FOR MRNA DELIVERY IN ARTICULAR JOINT CELLS.

Bettina Schwemberger[P][1,2], Lisa Sturm[1,2], Manish Aneja[3], Johannes Geiger[3], Johan Engbersen[4], Jaap Rip[4], Sri Dewi Hujaya[4], Mauro Alini[5], Andrea Traweger[1,2], Sibylle Grad[5], Valentina Basoli[5]

[1] *Institute of Tendon and Bone Regeneration, Paracelsus Medical University—Spinal Cord Injury & Tissue Regeneration Center Salzburg, 5020 Salzburg, Austria*

[2] *Austrian Cluster for Tissue Regeneration, 1200 Vienna, Austria*

[3] *ETHRIS GmbH, Semmelweisstrasse 3, 82152 Planegg, Germany*

[4] *20Med Therapeutics B.V. Drienerlolaan 5 - ZH252, 7522NB Enschede, The Netherlands*

[5] *Regenerative Orthopaedics, AO Research Institute Davos, Davos Platz, Switzerland*

Corresponding author's email: Valentina.Basoli@aofoundation.org

Tissues in the joint express a large amount of extracellular matrix (ECM) which interferes with transfecting agents. Current methods do not result in a satisfactory transfection of ECM-rich tissue. Biodegradable and bioresponsive polymer-based nanoparticles (Nanogel, 20Med Therapeutics) can be used as an oligonucleotide delivery technology and be optimized for transfection of specific cells. This makes it a promising candidate for in vivo delivery of therapeutic mRNA drugs. In this study, different transfection methods were evaluated in vitro for different cell types present in the synovial joint. Methods: Different concentrations of the Nanogel agent were tested besides Lipofectamine and NEON electroporation. Experiments were performed in monolayer culture on primary human bone marrow cells (hBMSC), human synovial stem cells (hSDSC), bovine chondrocytes (bCH), rat tendon stem/progenitor cells (rTDSPC). mRNAs were provided by Ethris GmbH. Successful transfection resulted in the translation of the respective fluorescent protein, which was evaluated by confocal microscopy and flow cytometry. Metabolic activity with AlamarBlue was performed after 24, 48, 72 and 96 hours. Results: Nanogel exposure did not affect cell viability as compared to lipofectamine and electroporation; however, the transfection efficiency varied based on the cell type. BMSCs and rTDSPC showed high protein expression for all the methods; on the contrary, bCH and hSDSC transfected with 30 µg/ml Nanogel and 480 ng mRNA showed a lower intensity in protein expression. Conclusions: Nanogel transfection proved successful for various cell types present in the joint and may be a promising method for tissues that exhibit a complex dense matrix.

Keywords

Transfection; Nanogel polymers; therapeutic mRNA

Abstract **#1298**

MICRORNA ENRICHMENT OF EXTRACELLULAR VESICLE CONTENT FOR DIABETIC WOUND TREATMENT POTENTIATION

Ricardo Abreu$_P$[1,2,3], Inês Albino[3], Marta Barão[3], Ana Branco[3], Hugo Fernades[4], Paula da Costa Martins[1,2], Lino Ferreira[3,4]

[1] *CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands*

[2] *Department of Molecular Genetics, Faculty of Sciences and Engineering, Maastricht University, Maastricht, Netherlands*

[3] *CNC- Centre for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*

[4] *Faculty of Medicine, University of Coimbra, Coimbra, Portugal.*

Corresponding author's email: rick.cerq.abreu@gmail.com

Extracellular vesicles (EVs) have been used for tissue regeneration in multiple contexts. However, their naïve potential may not be sufficient for clinical application (1). Here we investigate how to load small EVs with therapeutically active microRNAs. Different methodologies for loading EVs were tested (i.e., electroporation, saponin, cholesterol, freeze-thaw and Exo-Fect™) and, using a fluorescently-labelled microRNA, we showed that ExoFect™ was superior to the other methods (~83% transfection efficiency). The internalization kinetics of the modified EVs was evaluated using endothelial cells (ECs) and their bioactivity was evaluated, in vitro, using a reporter cell line for the presence of the exogenously loaded microRNA (2). Subsequently, sEVs were loaded with a miRNA capable of enhancing the survival of EC (identified using high-content screening) and topically administered, twice a day for 10 days, on wounds of STZ-induced diabetic mice. Our results showed that microRNA-loaded EVs were able to improve wound healing in diabetic mice. Importantly, analysis of wound tissue revealed increased expression of the delivered microRNA into the affected area and enhanced survival of ECs. In conclusion, we showed that Exo-Fect™ is an efficient and functional way of loading microRNAs into EVs. Furthermore, modulated EVs can be used in vitro and in vivo as effective delivery agents.

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Abstract **#1299**

NEURAL CELL GUIDANCE THROUGH TWO-PHOTON INDUCED DEGRADATION OF A BIFUNCTIONAL PHOTOCROSSLINKABLE HYDROGEL SYSTEM

Augusto Loffredo$_P$[1], Lana Van Damme[2], Jasper Van Hoorick[2], Sandra Van Vlierberghe[2,3], Abhay Pandit[1], Manus Biggs[1]

[1] *CÚRAM - SFI Research Centre for Medical Devices, National University of Ireland Galway, Galway, Ireland*

[2] *Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium*

[3] *Brussels Photonics, Department of Applied Physics and Photonics, Flanders Make and Vrije Universiteit Brussel, Brussels, Belgium*

Corresponding author's email: a.loffredo1@nuigalway.ie

Scaffolds that promote and sustain neuronal orientation and interconnectivity are of critical importance in regenerative strategies for the central and peripheral nervous systems. The biofabrication of scaffolds from materials with tuneable mechanical and physicochemical characteristics have the potential to mimic the microcellular 3D environment of native neuronal tissue [1]. Laser-based technologies have been applied in photoresponsive hydrogels to spatially tailor the aforementioned properties for a multitude of cell and tissue engineering applitactions [2]. In this study, a photocrosslinkable gelatin formulation was developed, with the aim to create an extracellular matrix mimetic construct with highly controlled spatiotemporal crosslinking properties [3]. By incorporating different moieties onto the free amines of the gelatin backbone, a bi-modular crosslinking mechanism was developed, resulting in a photoresponsive gel formulation capable of generating hydrogels with different degrees of stiffness through exposure with UV light. Furthermore, a femtosecond pulsed infrared laser was used in the presence of a biocompatible two-photon initiator [4] to induce microscale selective photodegradation/crosslinking of specific hydrogel regions. By creating 3D gelatin scaffolds possessing localised microscale regions of degradation/crosslinking, it was possible to initiate contact guidance induce neural cell alignment in a 3D hydrogel. In conclusion, we hypothesise that these physicomechanically patterned hydrogels can be used to fabricate tissue engineered neural conduits with selective spatial guidance.

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Abstract **#1300**

AN INJECTABLE SELF-HEALING HYDROGEL WITH CELL-MAGNETICALLY GUIDED AND GLUTATHIONE-CONTROLLED RELEASE FOR CARTILAGE REPAIR

Ling-Chun Chen[P][1], I-Yun Cheng[2], Jen-Hao Tsai[3], San-Yuan Chen[2], Shwu-Jen Chang[3]

[1] *Department of Biotechnology and Pharmaceutical Technology, Yuanpei University of Medical Technology*

[2] *Department of Materials Science and Engineering, National Chiao Tung University*

[3] *Department of Biomedical Engineering, I-Shou University*

Corresponding author's email: sjchang@isu.edu.tw

In this study, we proposed a self-healing injectable HA-pAA hydrogel integrated system with in-situ gelling, and semi-permanent for delivering cells engrafted with multifunctional porous PLGA magnetic microcarriers (PPMMs) was proposed for repairing articular cartilage tissue. The self-healing injectable HA-pAA hydrogel was synthesized through the mechanism of guest-host interaction using HA-cyclodextrin (CD) as host polymer and poly (acrylic acid)-ferrocene (pAA-Fc) as guest polymer. Owing to high specific surface ratio of porous structure, PPMMs could be loaded with 75.1% of GSH as reductant to improve anti-oxidation and anti-inflammatory ability as well as to induce hydrogels self-healing ability when suffering damage. Furthermore, the PPMMs with iron oxide nanoparticles loading and HA coating could bind with chondrocytes by CD44 binding site and magnetically chondrocytes were guided to damaged site through external magnetic force. The results showed that the PPMMs owing to its high specific surface ratio and stable sustained release within 24 hours. According to the rheological analysis, pAA-Fc with r = 4.0 could react with HA-CD to form an injectable self-healing hydrogel with a higher mechanical strength than other conditions. HA-pAA composite hydrogel could control the GSH release to achieve the various degree of self- healing and self-heal rapidly within 24 h when suffered external destruction. The chondrocytes encapsulated in the HA-pAA complex hydrogel could be guided by PPMMs to a specific injured place by magnetic guidance. The above-mentioned results demonstrate the application of self-healing injectable HA-pAA hydrogel integrated system is highly promising in cartilage tissue repair.

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Abstract **#1305**

PROTEOGENOMICS ANALYSIS OF ENDOCRINE DISRUPTORS ON AN IN VITRO HUMAN THYROID MODEL

Marta Nazzari$_P$[1], James Waddington[2], Orla Coleman[3], Duncan Hauser[4], Steven R Pennington[2,3], Florian Caiment[4]

[1] *Toxicogenomics, GROW School for Oncology and Developmental Biology, Maastricht University, 6229ET Maastricht, The Netherlands*

[2] *UCD Conway Institute, School of Medicine, University College Dublin, Belfield, Dublin 4, Ireland*

[3] *Atturos Ltd., c/o Conway Research Institute, University College Dublin, Dublin 4, Ireland*

[4] *Toxicogenomics, Maastricht University, 6229ET Maastricht, The Netherlands*

Corresponding author's email: m.nazzari@maastrichtuniversity.nl

As their name suggests, endocrine disruptors (ED's) are a class of chemicals able to interfere with the activity of the endocrine system. To date the effects of ED's on the transcriptome and proteome of the thyroid gland has not been extensively investigated and as a consequence data in public repositories are very scarce. In this study, we analyzed the transcriptome and proteome of human thyroid follicular epithelial cells, Nthy-ori 3-1, following exposure to several compounds representing different classes of ED's. The cytotoxicity of the ED's was also evaluated. Bis(2-ethylhexyl) phthalate (DEHP) was the selected compound for phthalates, triphenyl phosphate (TPP) for organophosphate flame retardants (OFR's), benzo[a]pyrene (B[a]P) for polycyclic aromatic hydrocarbons (PAH's), PCB-118 and PCB-153 for polychlorinated biphenyls (PCB's). None of the compounds resulted in a cytotoxic response at the concentrations tested after an exposure of 24, 48 and 72 hours. To generate a novel and comprehensive molecular picture of the impact of ED's on thyroid cells, RNA-sequencing (RNA-Seq) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were employed for the deep analysis of the transcriptome and proteome, respectively, following a 24 hours' exposure of Nthy-ori 3-1 cells to ED's. RNA-Seq analysis was performed using Combo-Seq, a method to capture and quantify both mRNA and microRNA from the same samples libraries. The latest results of this proteogenomics study will be presented.

Keywords

Thyroid; NGS; Proteomics

Abstract **#1306**

THE APPLICATION OF PULSED ELECTROMAGNETIC FIELD FOR CARTILAGE REGENERATION

Zheng Yang$_P$[1,2], Cenk Celik[2], Dinesh Parate[2], Alfredo Franco-Obregón[3,4,5], Eng Hin Lee[2,1]

[1] *NUS Tissue Engineering Program, Life Sciences Institute, National University of Singapore*

[2] *Department of Orthopaedic Surgery, National University of Singapore*

[3] *Department of Surgery, National University of Singapore*

[4] *BioIonic Currents Electromagnetic Pulsing Systems Laboratory, BICEPS, National University of Singapore*

[5] *Institute for Health Innovation & Technology, iHealthtech, National University of Singapore*

Corresponding author's email: dosyz@nus.edu.sg

Mesenchymal stem cells (MSCs) could participate in cartilage repair by direct differentiation to chondrogenic cells, or through the secretion of a plethoria of trophic factors, exerting paracrine function to the surrounding cells. MSCs are "environmentally responsive" to local micro-environmental cues and biophysical perturbations, including pulse electromagnetic field (PEMF) exposure. In this study, using an in-house designed PEMF delivery system producing precise magnetic field of low intensity and frequency, we demonstrate both augmentation of MSC chondrogenesis and secretome paracrine function by PEMF exposure. PEMF inductive effect are exquisitely dependent on the pulsing intensity and duration as well as the MSC culture platform (scaffold free pellet culture, hydrogel or fibrous scaffold culture). We further shown that PEMF directionality over the chondrogenic differentiation of MSCs laden on electrospun fibrous scaffolds of either random (RND) or aligned (ALN) orientations was governed by the interaction between magnetic field orientation and the cell micromechanical environment at the level of mitochondrial respiration. Further, PEMF-induced MSC secretome was capable of enhancing the migration of chondrocytes and MSCs as well as mitigating cellular inflammation and apoptosis. We provide evidence that brief exposure to low amplitude PEMFs enhanced the ability of MSCs differentiation, as well as secretory factors production capable of promoting cartilage regeneration as well as protecting against adverse inflammatory conditions. Collectively, our results indicate that PEMF stimulation could have broad clinical and practical ramifications for the enhancement and re-establishment of cartilage regeneration.

Abstract **#1307**

USE OF BIOHYBRID SILKOTHANE® FOR THE DEVELOPMENT OF COMPLIANCE-CALIBRATED SMALL DIAMETER VASCULAR GRAFTS

Alice Caldiroli[P][1], Elia Pederzani[2], Marco Pezzotta[2], Alessandro Caimi[2], Gianfranco Beniamino Fiore[2], Nadia Azzollini[3], Sonia Fiori[3], Matteo Tironi[3], Paola Rizzo[3], Fabio Sangalli[3], Marina Figliuzzi[3], Alberto Cesare Luigi Redaelli[2], Andrea Remuzzi[4], Monica Soncini[2], Stefania Adele Riboldi[1]

[1] *Bioengineering Laboratories S.r.l., Italy*

[2] *Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy*

[3] *Istituto di Ricerche Farmacologiche Mario Negri - IRCCS, Italy*

[4] *Università degli Studi di Bergamo, Italy*

Corresponding author's email: ACaldiroli@bioengineeringlab.com

The reduced radial compliance of synthetic grafts, with respect to native vessels, is considered one of their major causes of failure, especially in case of small diameter (D≤6mm). To address this issue, a novel semi-degradable three-layered vascular graft, composed of a blend of silk fibroin and polyurethane (Silkothane®) and manufactured by electrospinning, was previously developed and characterized (D≥6mm) [1-3]. The current study explores the possibility to employ such technology in the development of compliance-calibrated smaller prostheses (D~1.5mm). To this purpose, two clusters of grafts were electrospun: a stiffer one, composed of silk fibroin only (SF), and a more compliant one, enclosing a Silkothane® core (SF/PU). Their radial compliance was measured by means of a stereo microscope (Nikon, SMZ1000) equipped with a digital camera (Fire-I, UniBrain), evaluating the dimensional variation with respect to the applied pressure; three pressure ranges (50-90, 80-120 and 110-150 mmHg) were considered, according to ISO 7198 (Section A.5.9) [4]. SF grafts displayed compliance of approx. 1%/100mmHg, while SF/PU grafts around 2.5%/100mmHg. An in vivo evaluation is currently ongoing in a rodent model (rats), to verify whether differences in graft compliance determine different performances in terms of patency (presence of flow monitored by echo-doppler), platelet activation (evaluated via Platelet Activity State - PAS - assay), neointima formation and endothelialization (evaluated via histologic analyses).Two implantation sites (abdominal aorta and femoral arteriovenous shunt) and two explant time points (30 and 90 days) are being considered. Preliminary results are encouraging, since all implanted animals survived with blood flow inside the grafts.

Keywords

Hybrid vascular grafts; Compliance; Silk fibroin

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Abstract **#1308**

ENGINEERING PREVASCULARIZED COLLAGEN-BASED HYDROGEL TO ACCELERATE REGENERATIVE REPAIR OF VOLUMETRIC MUSCLE LOSS INJURY

Ting-Lun Hsu[P][1], Wei-Lin Chen[1], Shih-Yen Wei[1], Ying-Chieh Chen[1]

[1] *Department of Materials Science and Engineering, Tsing Hua University, Taiwan*

Corresponding author's email: whu930029@gmail.com

Volumetric muscle loss (VML) resulted from accidents or surgery, is associated with the irreversible loss of the muscle mass and function. In VML, the destroyed basal lamina resulted in the muscle cannot regrow without sufficient blood supplied by host vasculatures, even if skeleton muscle has an innate repair mechanism through activating satellite cells induced by the basal laminae and connective tissue. This leads to infiltrating fibroblasts dominate the regeneration, forming the functional scar tissue with mostly fibrosis, rather than muscle fiber regeneration.

Here, we aimed to develop collagen-based hydrogel that can tune a wide range of physicochemical properties to control engineered vascular network formation in vivo. Human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) laden hydrogels were implanted into subcutaneous space in nude mice to prepare high, intermediate and low degrees of cell-mediated prevascularized constructs by tailoring the crosslink degrees . After 7 days, the prevascularized tissue was implanted into the left rectus femoris muscle injury of mouse. After 1-month, large portion of repaired muscle with larger muscle fibers were well distributed at the injury site in the high prevascularized group. In contrast, more fibrosis and small muscle fibers were found in the intermediate and low prevascularized. Furthermore, to understand and highlight the importance of prevascularization, non-prevascularized cell-laden hydrogels were formed directly at the injury site. The results highlighted that prevascularized tissues accelerated muscle fiber repair through timely supply enough blood supply by pre-engineered blood vessels and avoided host fibroblasts invading, which assisted the repairment of VML.

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Abstract **#1309**

BIOPRINTING OF CORNEAL STROMA AND EPITHELIUM TISSUE WITH BIOMIMETIC HYDROGEL SUPPORTING SCARLESS CORNEAL TISSUE REGENERATION

Falguni Patiₚ[1], Shibu Chameettachal[1], Deeksha Prasad[2], Yash Parekh[3], Sayan Basu[2,4], Vivek Singh[2,4], Kiran Kumar Bokara[3]

[1] *Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Kandi, Sangareddy – 502285, Telangana, India*

[2] *SSR-Stem Cell Biology Laboratory, Center for Regenerative Ophthalmology, L V Prasad Eye Institute, Hyderabad, Telangana, India*

[3] *CSIR-Center for Cellular and Molecular Biology, ANNEXE II, Medical Biotechnology Complex, Uppal Road, Hyderabad, Telangana, India*

[4] *Centre for Ocular Regeneration (CORE), L.V. Prasad Eye Institute, Hyderabad, Telangana, India*

Corresponding author's email: falguni@bme.iith.ac.in

Impaired vision can affect a person's independence, education, employment, socio-economic status. As estimated by the World Health Organization (WHO), approx 1.3 billion people around the world are living with vision impairment. Corneal opacity is a leading cause of sight impairment worldwide, which results from scar formation due to infections from bacteria, fungi, viruses and protozoa or chemical injury or other accidental injuries. There have been few attempts to develop artificial corneal using both cell-based and matrix-based approaches; however, they have their own limitations. Recently, a few groups have tried to bioprint the corneal stroma; however, their corneal tissue regeneration potential is yet to demonstrate. We bioprinted corneal stroma and epithelium with decellularized cornea matrix (DCM) hydrogel that was developed by a novel protocol capable of preserving key ECM components while eliminating the maximum cellular contents. We also show the functionality of our bioprinted cornea tissue using various in vitro cellular assays and observed high cell viability, cellular migration, and maintenance of keratocyte morphology and function. We observe higher-order assembly of these cellular constructs with organized spatial patterns and tissue-specific gene expression. The most striking feature of the DCM hydrogel is its ability to prevent corneal scar tissue formation. The bioprinted cornea with DCM hydrogel has extraordinary translation potential for different corneal pathologies including traumatic injuries, subsequent scar formation, and even for regenerating entire cornea and thereby may eliminate the current dependency on the corneal donor tissue.

Abstract **#1310**

EXPLORING SEEDING METHODS TO EVENLY DISTRIBUTE CELLS IN A 3D ELECTROSPUN SCAFFOLD

Nicole Dvorak[P][1], Luka Savic[1], Sarah L Waters[2], Hua Ye[3], Andrew Carr[1], Pierre-Alexis Mouthuy[1]

[1] *Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Botnar Institute of Musculoskeletal Sciences, University of Oxford, Oxford, UK*

[2] *Mathematical Institute, University of Oxford, Oxford, UK*

[3] *Department of Engineering Science, Institute of Biomedical Engineering, University of Oxford, Oxford, UK*

Corresponding author's email: nicole.dvorak@ndorms.ox.ac.uk

The development of a novel flexible tissue engineering bioreactor requires optimal design and operating parameters. Initial uniform cell distribution is an important prerequisite as underpopulated areas can be a result of poor cell suspension but also nutrient deprivation and unsuccessful bioreactor design. Furthermore, cell distribution throughout the scaffold is often a design requirement of tissue-engineering products. To achieve even cell distribution, three seeding methods were tested for seeding efficiency. Several repeats of 3cm long electrospun PCL scaffolds were propped into three custom built stages and subsequently seeded with human hamstring cells, one with the scaffold filaments aligned along gravity, one aligned perpendicular to gravity, and one moved onto a rocking plate immediately after seeding. Cells were left to attach for 1 hr and subsequently fixed in 2.5% glutaraldehyde. Cell distribution was assessed using Osmium Tetraoxide stain and micro-CT analysis. Images were analysed using the Fiji 3D-Object counter to visualize cells above the threshold. Quantitative results were binned and plotted in a frequency distribution and heat map. SEM and Elemental x-ray analysis were used to confirm the findings. Results showed that cells readily adhere to the scaffold material and that seeding cells in a dynamic fashion greatly improved even cell distribution. The method of assessing this is a needed alternative for large tissue constructs as cryo-sectioning on large scale is time consuming and scaffold materials do not always withstand processing.

Keywords

cell distribution; scaffold design; imaging

Abstract **#1312**

DEVELOPMENT OF AN ORIGINAL SKELETAL MUSCLE CELLS CULTURE MODEL FROM HUMAN MUSCLE BIOPSIES

Antoine Florin[1], Cécile Lambert$_P$[1], Christelle Sanchez[1], Jérémie Zappia[1], Yves Henrotin[1,2]

[1] *MusculoSKeletal Innovative Research Lab (mSKIL), Arthropôle Liège, Center for Interdisciplinary Research on Medicines (CIRM) Liège, ULiège, Institute of Pathology, CHU Sart-Tilman, 4000 Liège, Belgium*

[2] *Department of physiotherapy and functional rehabilitation, Princess Paola Hospital, Vivalia, Marche-en-Famenne, Belgium*

Corresponding author's email: yhenrotin@uliege.be

Objectives.This work aimed to develop a culture model of primary skeletal muscle cells obtained from human muscle biopsies.Material and methods.Muscle biopsies were taken post-mortem from the vastus lateralis. The biopsies were enzymatically digested. Then, the satellite cells were purified by two methods: pre-plating or immunomagnetic cell sorting using an anti-CD56 antibody. The performance of these cell purification methods was evaluated by FACS. We also compared two proliferation media (10%FBS vs. 10%FBS + 2%Ultroser G (UG)) on the cell doubling time. Finally, we studied the expression of several muscle cell markers during myogenesis by qRT-PCR and immunofluorescence.Results.The cells obtained from the digestion of the biopsy contained varying proportions of CD56+ satellite cells (83.79 ± 11.88%). The immunomagnetic cell sorting method enable a higher enrichment with CD56+ satellite cells (95.59 ± 3.36%) than the pre-plating method (81.29 ± 7.76%). The proliferation media containing 10%FBS and 2%UG allowed to obtain a significantly shorter doubling time and than the proliferation medium containing only 10%FBS (25.46±4.9h vs. 70.19±22.9h, p=0.0286). Finally, the increase in MyoD1, myogenin and MYH demonstrated the phenotypic transition of our cells to a myogenic phenotype.Discussion.Immunomagnetic cell sorting allowed a better satellite cells purification than the conventionally preplating technique. UG serum substitute accelerated cell proliferation and improved the differentiation of myoblasts into myotubes. Conclusion.This culture model of human primary skeletal muscle cells could be helpful for the screening and the study of the mechanisms of action of compounds developed to treat muscle diseases like sarcopenia.

Abstract **#1313**

INTEGRATION OF 3D LUNG-ON-A-CHIP AND CONTROLLED AEROSOL INHALATION EXPOSURE SYSTEM FOR RECONSTITUTING LUNG PHYSIOLOGICAL FUNCTIONS AND ASSESSING THE TOXICITY OF PARTICLE MATTERS

Ko-Chih Lin[P][1]

[1] *Department of Electrical and Computer Engineering, College of Electrical and Computer Engineering, National Chiao Tung University, Hsinchu, Taiwan*

Corresponding author's email: tim1013.eed03@g2.nctu.edu.tw

Inhalation of particle matters (PMs) is the major route of PMs exposure and has led to adverse effects on the lung. It is of vast concern to evaluate the potential risks of PMs for human health during lung exposure. Here, we developed a human lung-on-a-chip integrating a newly aerosol exposure system to recreate the organ-level structure and functions of the human lung that allow us to assess the pulmonary toxicity of PMs under physiological respiratory airflow conditions in vitro. This novel lung-on-chip exposure platform was designed to connect up to 16 lung-on-chips and mimicked the range of flow rate of the human lung microenvironment. It cyclically inhaled microliter volumes of air through the upper epithelium-lined channel of the lung-on-chips with a programmable pump machine to regulate breathing behavioral parameters, including breathing frequency, and the inhalation duration, interval, and volume per channel, according to the expected values of human pulmonary. Furthermore, an aerosol generator exposed PMs into epithelial channels to observed the interaction of particles with critical cellular barrier functions at an air-liquid interface and assess the toxicity. Our biochip exposure systems not only highlight the prospects of realistic in vitro exposure tests in recapitulating typical local in vivo deposition results but also provide opportunities for advanced in vitro exposure tests for safety assessment for toxicity of particles, preclinical cytotoxicity, and drug screening applications.

Keywords

aerosol exposure system; lung-on-chips; microfluidic; particle matters

Abstract **#1314**

MODELING CHRONIC OBSTRUCTIVE PULMONARY DISEASE - INVESTIGATE THE EFFECT OF LC3B NUCLEAR TRANSPORT ON HUMAN LUNG FUNCTION AND DRUG RESPONDS IN VITRO

Shiue-Luen Chen[P][1], Hsiao-Chen Chou[2], Kuan-Chen Lin[3], Chong-You Chen[1], Guan-Yu Chen[2]

[1] *Department of Electrical and Computer Engineering, College of Electrical and Computer Engineering, National Chiao Tung University, Hsinchu, Taiwan*

[2] *Institute of Biomedical Engineering, National ChiaoTung University, Hsinchu, Taiwan*

[3] *Division of Hematology-Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan*

Corresponding author's email: g199500@gmail.com

Chronic Obstructive Pulmonary Disease (COPD) is an irreversible obstructive airway disease which is the third leading cause of death in the world. The possible causes are smoking, air pollution or industrial dust and chemicals. However, there is still no accurate prognosis, diagnosis and treatment strategy for COPD. Therefore, in vitro Establishing disease models with functions similar to the human body has become important. This research focuses on establishing an in vitro model of human small airway epithelium and remodeling human physiological behaviors, and found that the accumulation of LC3B nuclear import in COPD in the small airway play an important role in the differentiation model of cilia and mucous cells. In this study not only uses clinical drugs to regulate LC3B nuclear metastasis to improve the effect of COPD cilia differentiation also combine the high-throughput screening to analyze the lung function and drug responses. We found that Chloroquine (CQ) and Ivermectin (IVM) can be the treatment strategy for repairing cilia damage and mucus accumulation in COPD patients, and find the potential for analysis and treatment of COPD. On the other parts, we try to apply organ chip technology to reproduce the microenvironment of the small airways and bronchial tubes of the lungs to achieve personalized precision medicine.

Keywords

Small airways, Chronic Obstructive Pulmonary Disease, LC3B

Abstract **#1315**

MONITORING OF TISSUE MATURATION AND POLYMER ABSORPTION IN A RESTORATIVE POLYMERIC GRAFT AS A CAROTID INTERPOSITION IN A SHEEP MODEL

Julia Marzi[1,2,3], Eva Brauchle[1,2,3], Aurelie Serrero[4], Anton Bosman[5], Martijn Cox[4], Katja Schenke-Layland[1,2,3]

[1] *Department of Bioengineering, Faculty of Medicine, Eberhard Karls University, Tübingen, Germany*

[2] *Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies", Eberhard Karls University Tübingen, Germany*

[3] *NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*

[4] *Xeltis BV, Eindhoven, The Netherlands*

[5] *SupraPolix BV, Eindhoven, The Netherlands*

Corresponding author's email: julia.marzi@uni-tuebingen.de

Currently there are no good off-the-shelf solutions available for small diameter vascular applications due to limited patency of conventional synthetic grafts. Therefore, patients need to rely on their own veins, which are painfully harvested, mostly from the leg. A restorative graft, designed to be permeated by and replaced with the patient's own tissue through Endogenous Tissue Restoration (ETR) may help overcome current limitations. The ideal graft allows cellular infiltration leading to formation of functional neotissue, while the implant gradually absorbs. However, despite massive efforts in investigating new materials, up to date no restorative vascular graft is clinically available. Thus, there is an increasing demand for a better understanding of the mechanisms that direct graft regeneration or failure and for potential in situ tools to monitor these parameters.

The aim of this study was to investigate vascular remodeling processes in a restorative polymeric graft after 6 and 12 months implantation in a sheep carotid interposition model. Molecular and structural changes in biomaterial and tissue structures of the newly formed neointima were assessed by marker-independent and molecular-sensitive Raman microspectroscopy and imaging combined with multivariate analysis tools. In vivo polymer degradation was further compared to an in vitro degradation assay mimicking oxidative degradation. The biomaterial spectra revealed specific peaks that changed upon implantation duration and could be assigned to degradative processes originating from reactive oxygen species (ROS) present in the tissue. Imaging of the neointima allowed to identify and localize different extracellular matrix structures and determine the tissue maturation based on collagen I.

Abstract **#1316**

STEREOLITHOGRAPHIC FABRICATION OF CULTIVATION CHAMBERS FOR AXIALLY VASCULARIZED TISSUE-ENGINEERING

Nadine Nottrodt[P][1], Andreas Hoffmann[1], Benjamin Thomas[2], Hannah Helt[2], Ulrich Kneser[2], Wolfdietrich Meyer[3], Hadi Bakhshi[4]

[1] *Fraunhofer Institute for Laser Technology ILT*

[2] *BG Trauma Center Ludwigshafen*

[3] *Fraunhofer Institute for Applied Polymer Research IAP*

[4] *Fraunhofer-Institut für Angewandte Polymerforschung IAP*

Corresponding author's email: Andreas.Hoffmann@ilt.fraunhofer.de

Skin is the largest organ of every mammal and protects against sources of danger like hypothermia or dehydration. Skin defects are often caused by soft tissue trauma, burns, or surgical resections. Consequently, treatment efforts are aimed at restoring skin integrity on the one hand. Nevertheless, reconstruction of the underlying connective tissue is important for a full functionality of the tissue as well, on the other hand.

Therefore, new technologies are under investigation, allowing for the de novo engineering of connective tissue and skin within the patient's body. The arterio-venous (AV) loop technique represents one such promising approach. To this end, a vascular loop is generated microsurgically, placed inside a Teflon chamber, accompanied by a decellularized matrix, and subsequently implanted under the patient's skin for three to four weeks.

In the present study, we investigated new stereolithographic strategies to print patient tailored chambers. These newly designed chambers can help address several new properties: their geometry is tailored to the individual implantation site of the patient and their material is more elastic allowing for more comfort during the cultivation phase.

We investigated new classes of photopolymers, which are more elastic than Teflon and can be photo crosslinked. We tested them for their biocompatibility using 3T3-fibroblast cell culture and optimized the crosslinking protocols of the polymer using FTIR-analysis and improved the respective washing protocols. Furthermore, their mechanical properties and sewability are important for fixation of the chamber during the time of cultivation. Therefore, simple mechanical tests have been carried out.

Keywords

Stereoltihography; patient tailored; skin tissue cultivation

Abstract **#1317**

TAILORING THE PROPERTIES OF GELATIN METHACRYLATE (GELMA)/ALGINATE HYDROGEL BLENDS FOR BIOPRINTING OF SMOOTH MUSCLE CONSTRUCTS

Zongzhe Xuan[P][1], Lana Van Damme[2,3], Sandra Van Vlierberghe[3], Vladimir Zachar[1], Cristian Pennisi[1]

[1] *Regenerative Medicine Group, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*

[2] *Department of Plastic & Reconstructive Surgery, Ghent University Hospital, Ghent, Belgium*

[3] *Polymer Chemistry & Biomaterials Group, Center of Macromolecular Chemistry, Ghent University, Ghent, Belgium*

Corresponding author's email: zxuan@hst.aau.dk

The aim of the study was to investigate GelMA/alginate hydrogels blends for bioprinting of smooth muscle cells (SMCs). Our long-term goal is to engineer a platform to study the mechanisms that control the maturation of SMCs in the muscular layer of the urethra. Four hydrogel blends were prepared by mixing different concentrations (in %w/v) of GelMA/alginate: G1(5/1.5), G2(5/3), G3(7.5/1.5), G4(7.5/3). GelMA (10%) was used as control. The mechanical properties of the hydrogels, before and after crosslinking, were measured by rheometry. Ring-shaped constructs containing human bladder smooth muscle cells were fabricated using an extrusion-based printer. Following bioprinting, live/dead and metabolic assays were used to evaluate cell viability and proliferation. Rheometry analysis revealed that, in contrast to pure GelMA, addition of alginate significantly stabilized the variation in mechanical properties against changes in the temperature. The groups containing highest GelMA concentrations (G3, G4) displayed the largest viscosity, which, after crosslinking, resulted in constructs showing higher stability and lower swelling over time. The viability of the SMCs remained above 80% and was not significantly affected by the blend type. In all groups, cells adopted a spindle-like morphology and displayed a similar growth rate over a 5-day period. Overall, groups G3 and G4 appeared to offer better printability without compromising cell viability and growth. Although the optimal mechanical properties of the blends in terms of supporting optimal SMCs maturation still needs to be determined, the results of this work suggest that GelMA/alginate hydrogels constitute versatile and tunable hydrogels for smooth muscle tissue engineering.

Keywords

urethral tissue engineering; smooth muscle; bioink

Abstract **#1318**

OPTIMIZATION AND APPLICATION OF INTEGRIN-SELECTIVE LIGANDS TO GELLAN GUM HYDROGELS AS CELL TRANSPLANTATION VEHICLES FOR SPINAL CORD INJURY

Luís Rocha[1,2,3], Carles Más-Moruno[4], Eduardo Gomes[1,2], Rui Lima[1,2], Jonas Campos[1,2], Jorge Cibrão[1,2], Deolinda Silva[1,2,3], Andreas Räder[5], Horst Kessler[5], Rui Sousa[3], David Learmonth[3], António Salgado[P][1,2]

[1] ICVS - Life and Health Sciences Research Institute. Universidade do Minho, Braga - Portugal

[2] ICVS/3B's – PT Government Associate Laboratory, Braga, Portugal

[3] Stemmatters, Biotecnologia e Medicina Regenerativa SA, Barco, Portugal

[4] Department of Materials Science and Engineering Universitat Politècnica de Catalunya (UPC), 08019 Barcelona, Spain

[5] Technische Universität München, Department Chemie, Institute for Advanced Study, Lichtenbergstrasse 4, 85748, Garching, Germany

Corresponding author's email: id6526@alunos.uminho.pt

The biofunctionalization of otherwise essentially inert materials is usually done using linear adhesion peptides that partially lose their conformation upon binding, hence bioactivity, and without integrin specificity. In this work we addressed this issue and developed a gellan gum (GG) hydrogel modified with integrin-selective mimetics, aiming applying it as a promoter of the revascularization of spinal cord tissue following injury by locally implantating it with encapsulated ASCs and HUVECs. Indeed, faulting vascular repopulation is a key hallmark of spinal cord injury and decisively contributes to the poor prognosis associated to the condition.

Initially, different reaction stoichiometries were tested and characterized by varying the polymer:ligand ratio and the bioactivity of each condition tested and compared with GG functionalized with RGD employing the same ratios. The polymer grafted with the mimetic ligand in a certain ratio significantly promoted the adhesion of ASCs both at 3 and 7 days of culture. The same was observed even when reducing the GG:RGD ratio, proving that increased ligand specificity leads to enhanced bioactivity. The neurite extension from DRG explants was increased when combining the mimetic-functionalized hydrogel with cells (ASCs or ASCs+HUVECs). Preliminary in vivo data in a spinal cord injury transection rat model shows that the combination of modified GG and cells leads to a significant motor recovery, as well as to the decrease in pro-inflammatory serum markers during the acute phase of the condition. We show for the first time the potential of using integrin-selective ligands to enhance the bioactivity of hydrogels for CNS applications.

Abstract **#1319**

SURFACE VISCOSITY CONTROLS STEM CELL FATE

Eva Barcelona Estaje[1], Matthew Dalby[1], Pere Roca-Cusachs[2,3], Manuel Salmeron-Sanchez[1], Marco Cantini[1]
[1] *Center for the Cellular Microenvironment, University of Glasgow*
[2] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST)*
[3] *Department of Biomedicine, University of Barcelona*
Corresponding author's email: 2349210b@student.gla.ac.uk

The microenvironment where cells reside provides them with a range of cues that collectively affect their fate. These include biochemical cues, mediated by interactions with the extracellular matrix (via integrins) and between cells (via cadherins), and physical properties, such as stiffness and viscosity. While the effect of stiffness on cell fate has been widely studied, the role of viscosity has been only recently addressed (1). In our group, we demonstrated that cells sense viscosity using the same mechanotransductive mechanisms that they use for stiffness (2). Here, we investigate the role of viscous interactions in controlling stem cell fate. We designed mobile surfaces based on supported lipid bilayers of controlled viscosity, which target the adhesive crosstalk between integrins (RGD receptors) and cadherins (HAVDI-containing proteins) (3).
Using human mesenchymal stem cells, we observed an increase in cell adhesion and a decrease in actin flow when the viscosity of the surface and the concentration of RGD increased. When HAVDI was added to the surface, cell adhesion was reduced, while the actin flow increased. These changes provoked alterations in the activation of mechanotransductive pathways: the nuclear translocation of mechanosensitive YAP increased with viscosity and decreased with HAVDI ligation. Finally, early differentiation markers were differentially expressed, revealing an effect of viscosity on cell fate: while more mobile surfaces favored adipogenesis and HAVDI ligation on them triggered chondrogenesis, more viscous substrates promoted osteogenesis. Further investigations on the signaling pathways involved will allow to establish a paradigm to collectively understand and exploit cell response to viscous interactions.

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Abstract **#1321**

IN VITRO AND EX VIVO SCREENING OF SMALL MOLECULE THERAPEUTICS FOR OSTEOARTHRITIS

Zhen Li[P][1], Penghui Zhang[1], Valentina Basoli[1], Mauro Alini[1], Sibylle Grad[1]

[1] AO Research Institute Davos, Davos, Switzerland

Corresponding author's email: zhen.li@aofoundation.org

There is an urgent need to explore disease-modifying drugs for osteoarthritis (OA). Six small molecules (RCGD-423, Wogonin, XAV-939, Zingerone, THSG, and Paeonol) were thoroughly evaluated for their anabolic and anti-inflammation effects on 3D pellets of human OA chondrocytes in vitro.

3D pellets of human OA chondrocytes were cultured in chondrogenic medium for 7 days to generate cartilage tissue followed by small molecule treatment for 3 days within chondropermissive medium supplemented with 1 ng/mL IL-1β + 1 ng/mL TNF-α [1]. Compared with IL-1β+TNF-α group, Zingerone and Paeonol down-regulated MMP3 gene expression. Paeonol, XAV, THSG, and Wogonin up-regulated COL2 and ACAN gene expression. Wogonin, XAV, RCGD, Zingerone, and THSG down-regulated expression ratio of inflammatory/anti-inflammatory genes IL6/IL10 and IL8/IL10. At protein level, Zingerone, Wogonin, and XAV down-regulated IL-8 expression; RCGD up-regulated IL-10 expression. Compared with the pellets cultured in chondrogenic medium, pellets from the inflammatory model group did not show proteoglycan staining in extracellular matrix. Paeonol, Wogonin and XAV enhanced proteoglycan accumulation in pellets. To summarize all the factors evaluated, XAV and Wogonin showed the most pronounced anti-inflammatory and regenerative effect, as indicated by the highest positive effect factor numbers.

Human OA femoral heads were used to isolate osteochondral explants from the non-load bearing part. With the stimulation of IL-1β+TNF-α at different doses, a pro-inflammatory and catabolic effect was induced to mimic the OA condition. Explant OA models will be used to further evaluate the anti-inflammatory and regenerative effects of the most promising compounds ex vivo.

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Abstract **#1322**

3D BILAYER SPHEROID MODELLING OF THE MAMMALIAN CORNEA TO IDENTIFY NOVEL LIMBAL STEM CELL MARKERS

Melissa Jackson[1], Arianna Bianchi[1], Majlinda Lako[2], Colin Jahoda[P1]

[1] *Durham University*

[2] *Newcastle University*

Corresponding author's email: colin.jahoda@durham.ac.uk

Corneal diseases accounted for approximately 1,500,000 cases of blindness worldwide in 2015 (1). Yet whilst effective treatments are available, a key limitation is the availability of good quality epithelial cells that in vivo derive from the stem cell compartment, the limbus. Characterising the limbal niche is crucial for enabling the translation of novel therapies, but traditionally, this research uses primary mammalian corneal tissue due to the lack of a commercially available in vitro alternative.We have therefore developed a novel hanging drop spheroid model of the rabbit limbal cornea by surrounding stromal keratocyte (SK) cores with an envelope of limbal epithelial cells (LECs). Over 10 days, models were pulsed with EdU, cryosectioned and immunolabelled for key corneal markers or examined by transmission electron microscopy.Batches of up to 100 models were created with consistent cellular architecture. LEC envelopes showed discrete organisation and differentiation within four days of co-culture, characterised by the formation of a proliferative p63+, CK14+, vimentin+ basal population, with suprabasal and superficial LECs subsequently differentiating and upregulating CK12/3 and PAX6. The SK core adopted an in vivo phenotype, determined by the expression of vimentin and fibronectin, and negative expression of α-SMA. This data shows that our models recapitulate key limbal markers in both epithelium and stroma. Furthermore, LEC polarisation demonstrates the epithelial-stromal intercommunication taking place during model co-culture. Further work will develop our model as an in vitro high-throughput platform for use by the drug/cosmetics industry, and as a screening tool for selecting high-grade LECs suitable for transplantation.

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Abstract **#1323**

LARGE SKELETAL DEFECTS AND HOW TO HEAL THEM: IN SILICO MODELING OF THE INFLUENCE OF A BIOENGINEERED PERIOSTEAL MEMBRANE ON BONE REGENERATION

Laura Lafuente-Gracia[1,2], Aurélie Carlier[3], Liesbet Geris[1,2,4]

[1] *Biomechanics Section, Mechanical engineering department, KU Leuven, Belgium*

[2] *Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Belgium*

[3] *Department cBITE, MERLN Institute, Maastricht University, The Netherlands*

[4] *Biomechanics research unit, GIGA in silico medicine, University of Liège, Belgium*

Corresponding author's email: laura.lafuentegracia@kuleuven.be

Although a complete healing is possible after most bone fractures, there are adverse situations, such as large bone defects, where the conditions for the bone to heal are not present, leading to a non-union. In this study we investigated in silico the influence of a bioengineered periosteal membrane (coated with growth factors and/or cells and combined with oxygen releasing beads) as treatment strategy. An existing bioregulatory, oxygen-dependent fracture healing model [1,2] was used, capturing processes at the tissue, cell and intracellular scales. A large gap size was considered in the geometrical domain to simulate bone regeneration in a large skeletal defect. Several parameters sets were run in simulations of 90 days to capture delayed bone healing and non-union, where different scenarios were tested to analyze the influence of the periosteal membrane. These scenarios involved bioengineered membranes consisting of electrospun polycaprolactane meshes, combined with cells, growth factors and oxygen-related stimuli (either addition of oxygen releasing beads or by using hypoxia resistant cells). These simulations predicted successful healing for a number of the tested strategies. Although the current model lacks a description of the inflammatory phase and the implementation of different membrane geometries, this work provided a first insight for the identification of the most impactful in vivo conditions to be tested experimentally.

Keywords

bone regeneration; fracture healing; mathematical model

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Abstract **#1324**

VOLVOX CARTERI AS AN INNOVATIVE, VERSATILE, PROMISING BIOMATERIAL FOR TISSUE ENGINEERING

Mathilde STRICHER$_P$[1], Pascale VIGNERON[1], Claude-Olivier SARDE[2], Frédéric DELBECQ[2], Christophe EGLES[1]

[1] Université de Technologie de Compiègne, CNRS, Biomechanics and Bioengineering, Centre de recherche Royallieu – CS 60319 – 60203 Compiègne Cedex, France

[2] Université de Technologie de Compiègne, Ecole Supérieure de Chimie Organique et Minérale, Integrated Transformations of Renewable Matter (TIMR), Centre de recherche Royallieu – CS 60319 – 60203 Compiègne Cedex, France

Corresponding author's email: mathilde.stricher@utc.fr

Tissue engineering innovation has relied extensively and understandingly on animal products. Given the need for increasingly ethical, safe and economical alternatives, our postulate was to open up to vegetal matter seeking out tissue engineering concepts as cellular polarity, attachment, differentiation and extracellular matrix expansion. We found in the freshwater green alga Volvox carteri and its phylogeny a unique and global representation of this dimensional shift. Drifting from the unicellular alga Chlamydomonas reinhardtii, Volvox carteri colonies consist of an up to 500 µm spherical cohesive monolayer of ~ 2000 somatic cells surrounding a mucosal core, mainly composed of polysaccharides and hydroxyproline-rich glycoproteins holding progenitor cells.

Volvox carteri has displayed high stability and cellular adhesion capacities both on the surface and on extracts, offering multiple biomaterial shaping. Seeded Primary Normal Human Dermal Fibroblasts (HDFn) and Human umbilical vein endothelial cells (HUVECs) adhere to Volvox surface, forming cell carrier beads. Cellularized beads cultured for 21 days self-organize to develop three-dimensional dermis-like structures. Subcutaneous implantations of these pseudo tissue in a mouse model appeared stable and non-inflammatory in vivo even after 2 months.

Our data show how versatile and powerful Volvox-derived biomaterial can be in supporting eukaryotic cell growth as well as promoting self-organization. To our knowledge, our work represents the first step towards innovative use of volvocine algae as a bio-inspired material for tissue engineering, offering a wide range of applications from in vitro testing to regenerative medicine.

Abstract **#1325**

THERMORESPONSIVE SURFACE DESIGNED FOR PROLIFERATION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Akon Higuchi[1,2], Hangze Ruan[3]

[1] *National Central University*

[2] *WEnzhou Medical University*

[3] *Wenzhou Medical University*

Corresponding author's email: higuchi@cc.ncu.edu.tw

Thermoresponsive surfaces enable the detachment of cells or cell sheets by decreasing the temperature of the surface when harvesting the cells. However, human pluripotent stem cells (hPSCs), such as embryonic stem cells and induced pluripotent stem cells, cannot be directly cultured on a thermoresponsive surface; hPSCs need a specific extracellular matrix to bind to the integrin receptors on their surfaces [1-3]. We prepared a thermoresponsive surface by using poly(N-isopropylacrylamide-co-butylarylate), recombinant vitronectin and/or laminin-511 to provide an optimal coating concentration for hPSC culture. In particular, we developed a repeated and continuous cultivation method of hPSCs on the same thermoresponsive plates where hPSCs were partially released from the same thermoresponsive plates by lowering the temperature of the thermoresponsive plates below the LCST of the thermoresponsive copolymer. Subsequently, the residual cells on the thermoresponsive plates were repeatedly and continuously cultivated in fresh cultivation media, and the detached stem cells were continuously collected. These cultivation cycles (passages) of hESCs as well as hiPSCs were successively repeated for ten cycles in this investigation. The detached cells, even after continual culture for over ten passages, showed high pluripotency, the ability to differentiate into cells derived from the 3 germ layers in vitro (Embryoid body formation assay) and in vivo (teratoma formation assay) as well as the ability to undergo cardiac differentiation.

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Abstract **#1326**

MELT ELECTROWRITING FOR FABRICATION OF TEMPLATED MICROCHANNELS

Ali Nadernezhad$_P$[1], Matthias Ryma[1], Tomasz Jüngst[1], Jürgen Groll[1]

[1] *Chair for Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, University of Würzburg, 97070 Würzburg, Germany*

Corresponding author's email: ali.nadernezhad@fmz.uni-wuerzburg.de

Background and motivation: As a cheaper and more accessible alternative to the traditional photolithographic techniques, the templating methods based on 3D printing have become more popular in different areas of microfluidics and biofabrication research. However, the current 3D printing approaches fall short in generating delicate micro-structures in the range below 100 microns. In order to address this limitation, herein we present the first report on processing of water-soluble sugars by melt electrowriting (MEW). MEW provides the feasibility of direct writing for patterning with high flexibility in design and shape, making this method a powerful tool in fabrication of micron-scaled templating structures.

Methods: After evaluation of the thermal and physical properties of the sugar by DSC, TGA, and rheology, an operational window for MEW process was established and the influence of applied pressure, electrical field and tip-to-collector distance on the critical translation speed and the resulting fiber diameter were identified. Templating of the microchannels was achieved by embedding the printed structures in PDMS resin, followed by removal of the template through washing by water.

Results: It was successfully demonstrated that by fine-tuning the MEW process parameters during deposition of sugar-based microfibers, a wide range of structural resolution within a single design could be implemented. Our results show that complex designs with fiber diameters ranging between 10–200 µm were readily fabricated. Following a facile casting procedure, the resulting structures could be embedded within PDMS resin. A subsequent washing step resulted in a microfabricated PDMS devices with delicate perfusable internal structure.

Abstract **#1327**

OPTIMIZATION OF DECELLULARIZATION OF UTERINE TISSUE USING HIGH HYDROSTATIC PRESSURE

Dongzhe Wang[P][1], Narintadeach Charoensombut[2], Kinyoshi Kawabata[1], Tsuyoshi Kimura[3], Akio Kishida[3], Takashi Ushida[1], Katsuko Furukawa[1,2]

[1] *Department of Mechanical Engineering, University of Tokyo*

[2] *Department of Bioengineering, University of Tokyo*

[3] *Institute of biomaterial and bioengineering, Tokyo Medical and Dental University*

Corresponding author's email: dongzhe@g.ecc.u-tokyo.ac.jp

Scaffolds in organ reconstruction are vital as they provide a growth environment and mechanical supports for cells, and form the tissue structure. Compared to conventional synthetical scaffolds, a decellularized scaffold has advantages such as maintenance of structure and composition in the original extracellular matrix (ECM). Among many decellularization methods, high hydrostatic pressurization (HHP) stands out for its superior effects in ECM preservation. In the HHP method, due to the higher pressure tolerances of ECM than cells, tissue can be decellularized using an appropriate pressure condition. Otherwise, ECM may be disrupted or decellularization cannot be achieved. Moreover, unique structures and compositions in organs result in distinguished pressure resistance. These factors urge the optimization of pressure conditions for each specific organ. In this study, we optimized the pressure conditions by fabricating the decellularized scaffolds under different pressure and evaluating cell removal, ECM preservation, and in vivo regeneration. Results showed 250 MPa, 500 MPa, and 980 MPa can decellularize uterine tissue and retain ECM structural protein composition and mechanical properties. However, only 250 MPa scaffold presented regeneration of all layers in a uterine wall, especially the smooth muscle layer, after implantation. Thus, 250 MPa is considered as the optimal pressure of the HHP decellularization method for uterine tissue.

Keywords

High hydrostatic pressure; Uterus; Scaffold

Abstract **#1328**

DENTAL PULP STEM CELL-LADEN DECELLULARIZED BONE ECM/B-TCP COMPOSITE SCAFFOLD FOR DENTIN REGENERATION

Dongyun Kim[P][1], Miji Yeo[1], Jaeyoon Lee[1], Wonjin Kim[1], Jiun Lee[1], Hanjun Hwangbo[1], Soojung Chae[1], Jiyoung Hong[1], Juyeon Kim[1], Youngwon Koo[1], Hyeongjin Lee[1], GeunHyung Kim[1,2]

[1] *Department of biomechatronic Engineering, Sungkyunkwan University (SKKU), South Korea*

[2] *Biomedical Institute for Convergence at SKKU (BICS), Sungkyunkwan University, Suwon, 16419, South Korea*

Corresponding author's email: ehddbs6316@gmail.com

For the recovery of the severe damaged tissues or organs, artificial scaffolds have been fabricated using various biofabrication techniques. Among the techniques, bioprinting technology serves as a powerful tool for fabricating tissue in the field of tissue engineering. Since applying the proper bioink for successful bioprinting is important, naturally derived hydrogels such as collagen have been widely used in the field of study due to their biocompatible and physiological properties. However, there are still limitations to mimic the exact extracellular matrices (ECM)-like environment using the hydrogels. In this study, we prepared the bio-ink consisting of decellularized bone ECM (bECM), β-TCP, and human dental pulp stem cells (hDPSC). The composition of the bio-ink and bioprinting processes were optimized based on the cell viability and printability. Based on the optimized conditions, hDPSCs-laden bECM/β-TCP scaffolds (BTS group) were fabricated. To evaluate the cellular activities, collagen/β-TCP-based cell-laden structure (CTS) was chosen as a control group and various in vitro tests, such as MTT assay, immunofluorescence analysis, and RT-PCR were performed. According to the data, better cell proliferation was observed at the BTS groups. Moreover, the cells laden at BTS group showed improved expression of odontogenic differentiation markers (dentin matrix acidic phosphoprotein-1 (DMP-1), dentin sialophosphoprotein (DSPP), osteopontin (OPN)) than control groups. Therefore, we expected that this new bioink using ECM can be used for the research about hard tissue regeneration and has the potential for the tissue engineering field.

Keywords

decellularized bone ECM; cell-laden scaffold; dentin regeneration

Abstract **#1329**

BIOPRINTING OF CARDIOMYOCYTES USING A HYBRID COLLAGEN AND DECELLULARIZED EXTRACELLULAR MATRIX (DECM) HYDROGEL FOR HEART TISSUE REPAIR

Rabeil Sakina$_P$[1,2], Aida Llucià-Valldeperas[1,3], Carlos Mota[1], Paul Wieringa[1], Lorenzo Moroni[1]

[1] Complex Tissue Regeneration Department, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University, Universiteitsingel 40, 6229 ER, the Netherlands

[2] Department of Biomedical Engineering and Sciences, School of Mechanical and Manufacturing Engineering, National University of Sciences and Technology (NUST), Islamabad, Pakistan

[3] Department of Pulmonology and Physiology, Vrije Universiteit University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam, the Netherlands

Corresponding author's email: r.sakina@maastrichtuniversity.nl

Introduction

The development of ECM-derived hydrogels and their application in bioprinting remains challenging since disruption of ECM structure during decellularization can result in no hydrogel being formed (1). In this study, we propose the development of decellularized ECM hydrogels hybridized with collagen to enable cardiomyocyte embedded bioprinting in order to develop a physiologically functional myocardial construct.

Methods

Ventricular tissue was isolated from porcine hearts, cut into 1x1 mm2 cubes and treated with 1% (w/v) sodium dodecylsulfide (SDS) and 1% (v/v) triton at 4°C until the tissue was decellularized. DNA assay was done to analyze efficiency of cell removal. The decellularized tissue was then lyophilized and digested with pepsin to obtain 1.5% (w/v) ECM hydrogel. The ECM hydrogel was combined with 4mg/ml collagen gel solution in a 1:1 ratio to form the hybrid hydrogel. HL-1 cardiomyocytes were incorporated within the hybrid hydrogel. This 'bioink' was then extruded through a 0.2 mm internal diameter needle at 15-17 kPa and 15-25 mm/s. The constructs were either bioprinted using a grid (45°/90°) or a parallel fiber (80°/20°) design. Cell viability and proliferation was evaluated using a live/dead staining and DNA assay. Cardiac immunostainings were performed using antibodies against MEF2, SERCA2, α-actinin and cardiac troponin T. Gene analysis for cardiac specific genes was also conducted.

Results

The constructs were bioprinted upto 4 layers with fiber size 200 µm. Bioprinting of cardiomyocytes using the hybrid hydrogel showed good viability after 48 hours. Cardiac biomarkers were expressed in the bioprinted cardiomyocytes after two weeks of cell culture.

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Abstract **#1330**

GEOMETRICALLY CONTROLLABLE NANOSPIKES AS BIOACTIVE AND ANTIPATHOGENIC SCAFFOLDS FOR STEM CELL AND TISSUE ENGINEERING

Sunho Park[P][1], Yonghyun Gwon[1], Sujin Kim[1], Yubin Jeon[1], Hyoseong Kim[1], Jangho Kim[1]
[1] *Department of Rural and Biosystems Engineering, Chonnam National University*
Corresponding author's email: preference9330@gmail.com

Vertical slender nanostructure including nanopattern, nanowire, and nanospike, which can be directly interacted with living cells, have a great potential in biomedical applications. It was usually used as minimally invasive tools for drug delivery and intracellular sensing at the single cell level. Contrary to popular cognizance, we tried to investigate the potential of geometrically controllable nanospikes for regulating living cell behaviors including stem cells and bacteria cells. Therefore, we developed a nanospike patch with precisely controlled sizes using sophisticated nanoengineering (e.g., capillary force lithography). The structures of the stem cells were sensitively controlled by the nanospike, and the stimulation from the sharp corners induce the behaviors such as proliferation, differentiation, and cytokine excretion of stem cells. On the contrary, bacteria cells were damaged by the nanospike, and the antibacterial effects were promoted. In addition, stem cells integrated nanospike patches were easily engrafted onto the cranial bone defects, and the bone regeneration and calcium expression were significantly promoted by nanospike patches with stem cells. Likewise, geometry-controllable nanospike can play important roles as multifunctional biomaterials in the biological systems, and it can be used in the fields of tissue engineering and regenerative medicine for improving life of the living systems.

Keywords
Antibacterial; Stem cells; Nanostructure patch

Abstract **#1331**

DEVELOPMENT OF A 3-DIMENSIONAL VASCULARIZED CANCER MODEL VIA SOUND INDUCED MORPHOGENESIS (SIM)

Nicola Di Marzio[1], Preeta Ananthanarayanan[2], Géraldine Guex[1], Mauro Alini[1], Chiara Riganti[2], Tiziano Serra[1]

[1] *AO Research Institute Davos, 7270 Davos, Switzerland*

[2] *Department of Oncology, University of Torino, 10126, Torino, Italy*

Corresponding author's email: nicola.dimarzio@aofoundation.org

In the cancer microenvironment (CME) a heterogeneous cell population co-exist that communicates with the surrounding vasculature, inducing local neovascularization. In-vitro models recapitulating the in-vivo milieu are needed to evaluate new compounds and predict potential treatment effects in-vivo. Generating models which simulate the whole CME is challenging, and state-of-the-art 3D in-vitro vascularized cancer models have mostly been developed using microfluidics with limited high throughput scalability- which makes drug discovery long and expensive.

Using SIM technology in multi-well plates, we spatially organized human umbilical vein endothelial cells (HUVEC) into reproducible patterns. HUVEC rings (2 mm diameter, 100±50 µm thickness) were created in fibrin-gel in 60 seconds and self-assembled into a microcapillary network in 48h. A donor-specific heterotypic-cancer-spheroid, previously generated in a low-adherent 96-well plate, was superimpose on the microcapillary network and co-cultured for 4 days.

We investigated the presence of crosstalk among the model's elements and the effects of selected drugs on the system over 48 h. The microcapillary network's time-evolution, cancer spheroid sprouting and apoptosis were chosen as readouts, acquired by confocal microscopy and analyzed with imageJ. The microcapillary network area increased by 20% with an increase in branches of 60% compared to HUVEC-network alone, suggesting crosstalk between the microcapillary network and cancer spheroid. Following anticancer treatment, cancer spheroid sprouting decreased 75% and apoptosis increased 4-fold. Following antiangiogenic treatment, cancer sprouting decreased 45% but no increase in apoptosis was observed.

This platform offers an advanced in-vitro-model, compatible with high-throughput setups, in which spatially arranged microcapillary network co-exists with patient-specific cancer spheroid.

Abstract **#1332**

3D BIOPRINTING OF A CELL-LADEN THERMOGEL: AN EFFECTIVE TOOL TO ASSESS DRUG-INDUCED HEPATOTOXIC RESPONSE

Sara Maria Giannitelli[P][1], Manuele Gori[2], Miranda Torre[1], Pamela Mozetic[3], Franca Abbruzzese[1], Marcella Trombetta[1], Enrico Traversa[4], Lorenzo Moroni[5], Alberto Rainer[1,3]

[1] *Department of Engineering, Università Campus Bio-Medico di Roma*

[2] *Laboratory for Regenerative Orthopaedics, Department of Orthopaedic Surgery and Traumatology*

[3] *Institute of Nanotechnology (NANOTEC), National Research Council*

[4] *School of Energy Science and Engineering, University of Electronic Science and Technology of China*

[5] *MERLN Institute for Technology Inspired Regenerative Medicine, Department of Complex Tissue Regeneration*

Corresponding author's email: s.giannitelli@unicampus.it

Recent advances in bioprinting techniques have led to the investigation of novel hydrogel formulations to overcome the extensive in vitro culture required by the seeding of prefabricated 3D scaffolds. In this framework, the combination of different materials arises as a promising route to develop hydrogel matrices with cytocompatible gelation mechanisms and tailored chemo-mechanical behavior[1]. Thus, tunable properties can be achieved through the integration of multiple solidification/gelling mechanisms and multi-step cross-linking systems[2–4].

Here, a thermoresponsive Pluronic/alginate semi-synthetic hydrogel is used to bioprint 3D hepatic constructs, with the aim to investigate liver-specific metabolic activity of the 3D constructs compared to traditional 2D cultures[5]. A novel method for bioprinting hepatic cells is presented, via a robust and reproducible manufacturing process, characterized by high-shape fidelity, mild depositing conditions and easily manageable gelation mechanism. Furthermore, the dissolution of the sacrificial Pluronic templating agent significantly ameliorates the diffusive properties of the printed hydrogel.

The findings herein demonstrate high viability and liver-specific metabolic activity, as assessed by synthesis of urea, albumin, and expression levels of the detoxifying CYP1A2 enzyme of cells embedded in the 3D hydrogel system. A markedly increased sensitivity to a well-known hepatotoxic drug (i.e. acetaminophen) is observed for cells in 3D constructs compared to 2D cultures. Therefore, the developed 3D model may represent an innovative in vitro platform, alternative to in vivo tests, for investigating drug-induced hepatotoxicity.

Keywords

drug hepatotoxicity; 3D liver models

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Abstract **#1333**

CELLULOSE AEROGEL FIBERS FOR WOUND DRESSING APPLICATIONS

Matin Rostamitabar$_P$[1], Gunnar Seide[1], Stefan Jockenhoevel[1,2], Samaneh Ghazanfari[1,2]

[1] *Aachen-Maastricht Institute for Biobased Materials, Faculty of Science and Engineering, Maastricht University, Geleen, The Netherlands*

[2] *Institute of Applied Medical Engineering, RWTH Aachen University, Aachen, Germany*

Corresponding author's email: matin.rostamitabar@maastrichtuniversity.nl

Cellulose aerogel microfibers as a biobased material with high porosity, low density, and high specific surface area are suitable for various biomedical applications including wound dressings. The high viscous solution of the cellulose (9% w/v) in ZnCl2 molten salt hydrate was spun in two different diameters (100 and 300 µm) by a customized wet spinning line at 70 oC and regenerated in isopropanol. The salt-free wet spun alcogel fibers were dried and loaded with model drugs using supercritical CO2 (sCO2), and the white opaque cellulose aerogel fibers with surface area in the range of 100-160 m2/g were fabricated. Scanning electron micrographs of the cross-section of the aerogel fibers showed macro-porous outer shell with a nano-porous inner core. Fourier transforms infrared spectroscopy and X-ray diffraction analyses confirmed that the spinning and sCO2 drying of microcrystalline cellulose did not change the cellulose structure but weakened the inter- and intra-cellulose hydrogen bonding, and thereby disrupted the final aerogel fiber crystalline formation. The fibers absorbed moisture up to 15% weight ratio in the relative humidity of 80% over 24 hours mainly due to cellulose hydroxyl groups and microfibers' porous structure. Finally, drug-loaded fibers with specified morphological characteristics containing different model drugs and non-loaded fibers to provide thermal insulation and drainage of wound exudate were needle punched as a nonwoven aerogel textile. This new method of fiber fabrication can provide a novel concept in the design of the multi-functional wound dressings.

Keywords

Cellulose aerogel fibers; Supercritical drying; Wound dressing

Abstract **#1334**

EARLY REMOVAL AND REDUCTION OF DECONTAMINATION AGENTS.

Takashi Kakimoto[P][1], Koki Abe[1], Isao Tanaka[1], Takashi Kurihara[1], Kengo Tomita[1]

[1] *SHIMIZU CORPORATION*

Corresponding author's email: kaki-t@shimz.co.jp

Cell processing facilities, decontamination with chemicals is performed on a regular basis to prevent contamination. This is a very important task to keep the cell processing facility clean. However, there have been reports of cases where the residual decontamination agent affects cultured cells and workers. We report a decontamination agent adsorption experiment using a chemical filter for the purpose of early removal and reduction of residual decontamination agent removal.

Keywords

cell processing facility; decontamination

Abstract **#1336**

STIFF AND SOFT COATINGS, AS A POTENTIAL NEW ANTIMICROBIAL STRATEGY FOR BIOMATERIALS

Annabelle Vigué[1], Dominique Vautier[1], Julie Hardouin[2], Youri Arntz[1], Vincent Ball[1], Thierry Jouenne[2], Bernard Senger[1], Lydie Ploux[1]

[1] *U1121, Biomaterials Bioengineering INSERM/University of Strasbourg, France*

[2] *PBS laboratory, CNRS/University of Rouen, France*

Corresponding author's email: ploux@unistra.fr

Fighting microbial biofilms on biomaterials is usually addressed by incorporating antimicrobial agents. Nevertheless, as usual in the natural life, intrinsic properties of the material surface can also be a complementary approach. They may reduce the quantity of adhered microorganisms and the remaining microorganisms can be treated with classical drugs. Mechanical properties of materials recently emerged as a possible way to impact biofilm formation. However, many questions have still to be elucidated so far. Roles of coating hydration, bacterial motility on the surface or the involvement of adhesion proteins and structures in the biofilm development on such coatings are especially questioned.

In this context, we investigated whether hydrogel and non-hydrogel soft and stiff films may differently impact microbial adhesion, motility and further biofilm formation. The films have been characterized in terms of viscoelasticity, hydration and chemistry, and microbial mobility, adhered quantity and production of biological structures for adhesion have been specifically investigated. The study has been conducted with yeast (Candida albicans) and bacteria species (Escherichia coli) as models, and by using fluorescence confocal microscopy and proteomic approaches.

Our results reveal that the stiffness differently impacts the amount and mobility of the adhered cells according to the nature of the film. The softness- and hydration-dependent microbial phenomena also vary with bacteria and yeast species. Finally, this may confirm the relevance of using some soft coatings to prevent biofilm formation on a material but also clarifies the risks to get opposite effects as desired if other crucial surface properties have not been associated.

Abstract **#1337**

COMPOSITE BLENDS FOR BONE TISSUE ENGINEERING: SYNTHESIS AND BIOLOGICAL EVALUATION

Maria Chatzinikolaidou[P][1,2], Rafaela-Maria Kavasi[1,2], Georgia-Ioanna Kontogianni[1], Raasti Naseem[3], Kenneth Dalgarno[3], António Azevedo[4], Paulo Quadros[4], Giorgia Montalbano[5], Ilaria Corvaglia[5], Sonia Fiorilli[5], Chiara Vitale Brovarone[5]

[1] *University of Crete*

[2] *FORTH-IESL*

[3] *Newcastle University*

[4] *FLUIDINOVA*

[5] *Politecnico di Torino*

Corresponding author's email: mchatzin@materials.uoc.gr

Osteoporosis, a bone pathology with a high socio-economic impact is rapidly increasing due to an ageing population. The GIOTTO project [1] aims to develop a medical device to treat osteoporotic fractures in long bones. Here, we report on the materials designed, fabricated and biologically evaluated. The materials include poly(ε-caprolactone) (PCL), poly L-lactic acid (PLLA), blends of PLLA, PCL, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (90:5:5%wt), and hybrid blends containing (5% w/w) strontium-substituted nano-hydroxyapatite (Sr-nano-HA) produced by wet chemical precipitation. For the blended materials the blend ratios were selected on the basis of the resulting mechanical properties. All materials were melt processed by extrusion into 1.75 mm diameter filaments. The osteogenic potential of the pre-osteoblastic cells cultured with filaments was evaluated by determining the alkaline phosphatase (ALP) activity, the secretion of collagen, the matrix biomineralization, and the gene expression of the osteogenic markers ALP, bone sialoprotein (BSP-1) and collagen I (Coll-a1). Our results demonstrate that all of the materials are not genotoxic and support a strong cell adhesion, viability and proliferation of pre-osteoblastic cells. The alkaline phosphatase (ALP) activity levels show significantly higher values for the blend containing Sr-nano-HA for all time points, while all other materials show similar levels to TCPS. A significant gene expression of ALP, BSP-1 and Coll-a1 was observed. All materials indicate high levels of secreted collagen with PLLA indicating a significant increase. The latter results in combination with the high levels of calcium deposition in all materials provide solid evidence of the formation of a healthy mineralized extracellular matrix.

Keywords

bone tissue engineering; osteogenesis

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Abstract **#1338**

PHOTO-RESPONSIVE AND ENZYMATICALLY DEGRADABLE CLICK HYDROGELS FOR VASCULARIZED GRAFTS

Muhammad Anwaar Nazeer$_P$[1,2], Syeda Rubab Batool[3], Seda Kizilel[1,2,3]

[1] *Chemical and Biological Engineering, Koç University, Sariyer 34450, Istanbul, Turkey*

[2] *Research Center for Translational Medicine, Koç University, Sariyer 34450, Istanbul, Turkey*

[3] *Biomedical Sciences and Engineering, Koç University, Sariyer 34450, Istanbul, Turkey*

Corresponding author's email: mnazeer13@ku.edu.tr

One of the key challenges in engineering three-dimensional tissue constructs is the creation of a mature microvascular network capable of supplying sufficient oxygen and nutrients to the tissue which makes ready-to-use vasculature a serious need in clinical treatments. The goal of this work is to investigate vascularization potential within a polyethylene glycol (PEG) based click hydrogel in vitro. Our functional hydrogel has two properties: i) it can degrade in response to matrix metalloprotease (MMP) enzymes due to the conjugated enzymatically degradable peptide within the network, and ii) can promote angiogenesis within the network through induced pluripotent stem cell-derived endothelial cells (iPSC-ECs). Our hydrogel retains covalently conjugated KLTWQELYQLKYKGI (QK) peptide sequence through a light cleavable o-Nitrobenzyl (ONB) linkage. QK peptide sequence can be released upon demand with a light exposure to manipulate the process of vascularization and has been established to promote vascularization. Our hydrogels were synthesized with 4-arm PEG-Azide and 4-arm PEG-DBCO through strain-promoted azide–alkyne cycloaddition (SPAAC) reaction at physiological temperature in the presence of ONB-containing QK-Azide and degradable peptide. Synthesis of materials and hydrogel was verified through advanced characterization techniques such as attenuated total reflection-Fourier-transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, scanning electron microscopy and others. Our design will be of significant value for cardiovascular diseases and cell transplantation technology in regenerative medicine.

Abstract **#1340**

MIXED POLYMER AND BIOCONJUGATE CORE/SHELL ELECTROSPUN FIBRES FOR BIPHASIC PROTEIN RELEASE.

Inchirah Adala$_P$[1], Jopeth Ramis[2], Mahetab Amer[2], Andrew Bennett[3], Cameron Alexander[3], Felicity Rose[3]

[1] *Phd student*

[2] *Post doc*

[3] *Supervisor*

Corresponding author's email: Inchirah.ADALA1@nottingham.ac.uk

Single protein delivery systems are oversimplified and do not represent the physiological environment. Biphasic protein release strategies will allow electrospun scaffolds to meet the varied demands of the different phases of regeneration within in vivo environments.1Therefore, the need to develop multiple protein delivery systems in tissue engineering has become a fundamental and urgent need. However, finding a suitable biomaterial and an encapsulation technique that are compatible for the release of multiple proteins is challenging.2-3In this study, proteins were physically and chemically loaded into a single coaxial electrospun fibre scaffold to obtain bi-phasic release profiles.Cyto-compatible polymers were used to construct the scaffold, using polyethylene oxide(PEO) for the core and polycaprolactone (PCL) reacted or mixed with Jeffamine (JFA) for the shell. Horseradish peroxidase, a model protein, was loaded in the core and functionalised onto the scaffold surface by coupling of protein carboxyl groups to the available polymer amine groups. The electrospun fibre scaffolds generated by reacting PEO/PCL with 1,6-diaminohexane and those from mixing PEO/PCL with JFA were further characterised for protein conjugation and release.Fibres prepared by the mixed PEO/PCL/JFA system were found to be the most appropriate for the simultaneous release of protein from the core and the immobilisation of another protein on the shell of the same scaffold.Moreover, JFA enhanced scaffold properties in terms of porosity and elasticity.Finally, we successfully demonstrated the cytocompatibility and the response to the protein-loaded scaffold using HepG2 cells by assessing cell attachment and metabolism respectively to a bovine serum albumin-conjugated scaffold and a retinoic acid-loaded scaffold.

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Abstract **#1341**

REGENERATION OF CARTILAGE THOUGH ACTIVATION OF TISSUE RESIDENT SKELETAL STEM CELLS AND AUGMENTATION OF THE NICHE

Matthew P. Murphy[P][1,2], Lauren S. Koepke[2], Michael T. Lopez[2], Xinming Tong[2], Thomas H. Ambrosi[2], Gunsagar Gulati[2], Owen Marecic[2], Yuting Wang[2], Ryan C. Ransom[2], Malachia Hoover[2], Michael T. Longaker[2], Charles K.F. Chan[2]

[1] *University of Manchester*

[2] *Stanford University*

Corresponding author's email: matthew.murphy@ucdconnect.ie

Introduction: Osteoarthritis (OA), the most common form of arthritis, is a major global health burden. Microfracturing (MF), a technique used to treat patients with OA results in a transient improvement of symptoms and the formation of fibrocartilage. We do not know what effects MF have on the Skeletal Stem Cell (SSC).

Aims: 1) To investigate the effect on MF on SCC 2) Augement the SSC niche to regenerate cartilage in a mouse model 3) Develop a translational xenograft model.

Materials and Methods: Animals- Jaxx mice b-Actin Cre-ER, R26Rconfetti, C57BL/6J, NSG. Human tissue: adult femoral heads, foetal phalangeal bones. Methods: FACS, microarray, CFU, EdU, in vitro culture, in vivo models: MF, DMM, renal capsule transplants, parabiosis, xenograft. Statistical analysis-Prism Software: Ordinary one way ANOVA test with Sidak post hoc analysis.

Results: Validated in mouse and human tissue: With age there is a significant ($p<0.001$) reduction in resident SSC. Acute injury results in a significant ($p<0.0001$) local increase in resident SSC and a significant ($p<0.012$) increase in proliferation of resident SSC. SSC intrinsically change in their in vivo differentiation capacity (renal capsule and orthotopic) and gene expression (microarray) following acute injury. Augmentation of the niche with BMP2 and VEGFr1 regenerates stable hyaline-like cartilage confirmed on histology (IF and Pentachrome) and Atomic Force Microscopy.

Abstract **#1342**

EXPLORING GLYCOSAMINOGLYCAN DISTRIBUTION IN CARTILAGE TISSUES USING CONTRAST-ENHANCED MICRO-COMPUTED TOMOGRAPHY

Manuela A Boosᴘ[1], Manula SB Rathnayake[1], Kathryn S Stok[1]
[1] *Department of Biomedical Engineering, The University of Melbourne*
Corresponding author's email: mboos@student.unimelb.edu.au

Development of functional tissue-engineered constructs benefits from non-destructive longitudinal imaging methods to assess ECM production. Contrast-enhanced micro-computed tomography (CECT) has emerged as a novel technique for 3D visualisation and longitudinal assessment of glycosaminoglycan (GAG) content in articular cartilage tissue-engineered constructs [1]. However, it has not been used for structures with a different ECM composition to articular cartilage; e.g. auricular cartilage. This study aims to test the feasibility of CECT for auricular cartilage compared to articular cartilage and investigate the influence of ECM composition.Bovine cartilage plugs (ø5 mm) from knees and ears were incubated in 12 mgI/ml CA4+ and scanned at 4µm using microCT. Total GAG content was assessed using DMMB assays. GAG distribution was evaluated using microCT, Safranin-O histology, and immunohistochemistry.GAG content normalised to volume shows a significant difference between articular cartilage (14.17±3.27 µg/mm3) and auricular cartilage (27.83±5.21 µg/mm3). Furthermore, mean attenuation is significantly higher in auricular cartilage (2.15±0.12 cm-1) compared to articular cartilage (1.31±0.14 cm-1). MicroCT images match the attenuation profiles. CA4+ shows significantly slower diffusion in auricular cartilage (τ=1.51) compared to articular cartilage (τ=0.78). In this study, CECT was successfully used to assess GAG distribution and content in auricular cartilage. CECT has potential for non-destructive longitudinal assessment and 3D visualisation of ECM production. The results suggest that incubation times should be adapted depending on tissue types and if attenuation is being compared to DMMB results, care must be taken to normalise results for different tissue densities.Thanks to Professor Mark Grinstaff, Boston University, for the CA4+.

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Abstract **#1343**

MIMICKING THE GAG RICH DOMAINS OF CARTILAGE TISSUES TO RECAPITULATE MECHANICAL BEHAVIOUR

Jemima E Schadow$_P$[1], Manuela A Boos[1], Brooke L Farrugia[1], Kathryn S Stok[1]

[1] *Department of Biomedical Engineering, The University of Melbourne*

Corresponding author's email: jeschadow@student.unimelb.edu.au

There is recent evidence that beyond bulk mechanics driving cell response, microstructural features regulate tissue-to-cell mechanotransduction in cartilage. Glycosaminoglycans (GAGs) are well known for their load-bearing properties, and there is significantly different viscoelastic behaviour in two cartilage types due to heterogeneity of GAG and ECM fibres [1]. Understanding how the mechanical microenvironment influences cellular response and cell-material interaction is important for functional tissue-engineering. This study aims to incorporate chondroitin sulphate (CS) in gelatin methacrylate (gelMA), a widely used hydrogel in tissue-engineering, at tuneable concentrations. This will allow for customising GAG-rich domains in cartilage tissues, and will enable investigation of the interaction of cells with the ECM in tissue-engineered constructs.10%w/v gelMA was enriched with up to 10% CS using reductive amination and crosslinked using Irgacure2959. The resulting hydrogels were characterised for sol fraction, macromere retention, swelling ratio, and mechanics. Sol fraction and macromere retention show successful incorporation of CS in gelMA at different concentrations. The resulting swelling ratio of gelMA hydrogels with CS is larger compared to gelMA alone. Furthermore, gelMA with CS shows different mechanical properties compared to gelMA alone.In this study, CS was successfully incorporated in gelMA using reductive amination. The resulting hydrogel characteristics resemble the load-bearing properties of native cartilage. This allows for fabrication of 3D hydrogel microenvironments with tuneable CS that mimics the heterogenous ECM in cartilage; an optimal platform for the investigation of mechanobiological processes. Further studies will shed light on the role of GAGs in mechanotransduction and reveal cellular responses to mechanical cues.

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Abstract **#1344**

BIOCOMPATIBILITY ASSESSMENT OF PURE AND STRONTIUM-SUBSTITUTED NANO-HYDROXYAPATITE FOR BONE REGENERATION

Maria ChatzinikolaidouP[1,2], Georgia-Ioanna Kontogianni[1], Rafaela-Maria Kavasi[1,2], António Azevedo[3], Paulo Quadros[3], Giorgia Montalbano[4], Sonia Fiorilli[4], Chiara Vitale Brovarone[4]

[1] *University of Crete*

[2] *FORTH-IESL*

[3] *FLUIDINOVA, S.A.*

[4] *Politecnico di Torino*

Corresponding author's email: mchatzin@materials.uoc.gr

We report on the development of novel biomaterials containing pure as well as strontium-substituted nano-hydroxyapatite (nano-HA) within the GIOTTO project [1] that aims to target specific osteoporotic fractures using innovative customized therapeutic devices. Both, the pure hydroxyapatite forms nano-HA_powder and nano-HA_paste, as well as the 50 and 100% strontium-substituted hydroxyapatite (Sr-nano-HA_50 and Sr-nano-HA_100, respectively) were produced by wet chemical precipitation in the NETmix® reactor (FLUIDINOVA S.A.), using calcium, strontium and phosphorous salts as starting materials. The slurries obtained were washed to remove the unreacted remaining salts and final aqueous pastes were concentrated up to 15% wt. Each material was diluted to a final concentration of 0.25% v/v and cultured with pre-osteoblastic cells for the evaluation of the cell viability, genotoxicity, alkaline phosphatase (ALP) activity, calcium biomineralization, collagen deposition and expression profile of the osteogenic genes ALP, bone sialoprotein (BSP-1) and collagen type I (Coll-a1). All materials are not genotoxic, show high cell viability and indicate a higher ALP activity on days 3 and 7, with the Sr-nano-HA_50 showing a significant increase. Calcium mineralization was higher on all materials compared to the control after 7 days in culture. Collagen secretion was high in all nano-HA materials, with the nano-HA_powder showing significantly higher collagen secretion after 14 days. Real-time quantitative polymerase chain reaction showed that the expression of bone sialoprotein (BSP-1) and collagen type I (Coll-a1) was significantly higher on nano-HA_powder, and higher than the control on all other materials. These results indicate a high osteogenic potential of the nano-HA materials.

Keywords

biomaterial; osteoporosis

References

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Abstract **#1345**

THE INCORPORATION OF A NATIVE-LIKE EXTRACELLULAR MATRIX IN NERVE GUIDE CONDUITS FOR PERIPHERAL NERVE REPAIR

Sara Memarpour Hobbi[1,2], Simon Atkins[1], Frederik Claeyssens[3,2], John Haycock[3,2], Fiona Boissonade[3,1]

[1] *School of Clinical Dentistry, University of Sheffield, Sheffield, S10 2TA*

[2] *Kroto Research Institute, North Campus, University of Sheffield, Sheffield, S3 7HQ*

[3] *University of Sheffield*

Corresponding author's email: smemarpourhobbi1@sheffield.ac.uk

The repair of peripheral nerve injuries is limited by current clinically available nerve guide conduits (NGCs), which do not provide adequate levels of regeneration compared to the gold-standard of a nerve autograft.

Studies suggest that the use of individual extracellular matrix (ECM) components can improve nerve regeneration (1), yet fewer studies have investigated the effect of incorporating a more native-like extracellular matrix as a scaffold for nerve regeneration, containing all the components present in a peripheral nerve.

The current project aimed to incorporate a neuronal native-like ECM in NGCs by using chick dorsal root ganglia, a source of primary nerve tissue that contains neuronal, glial and non-glial cells within the ganglia.

The cellular components were removed by decellularisation, resulting in a naturally-obtained decellularised ECM maintaining features of the native peripheral nerve tissue. The decellularised ECM showed improved cell attachment, neurite extension and neurite length compared to control tissue culture plastic, highlighting the potential for a naturally cell-derived ECM in nerve repair.

In order to further support the deposition of ECM, electrospun microfibres can be used as intraluminal scaffolds within nerve guide conduits, providing both physical and chemical cues to regenerating nerve fibres across injuries. Nerve guide conduits were fabricated from photocurable polycaprolactone by microstereolithography, while aligned polycaprolactone fibres were produced by electrospinning and plasma treated with oxygen. Nerve regeneration was assessed in vitro using a relevant neuronal NG108-15 cell line.

Future studies will assess regeneration in vivo using the transgenic YFP mouse line with fluorescent nerve fibres.

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Abstract **#1346**

DEVELOPING BIMODAL PET/MRI IMAGING AGENTS FOR IN VIVO CELL TRACKING

Niamh E. McCallum$_P$[1,2,3], Patricia Murray[1], Matthew J. Rosseinsky[2], Harish Poptani[3]

[1] Department of Molecular Physiology and Cell Signalling, Institute of Systems, Molecular and Integrative Biology, University of Liverpool

[2] Department of Chemistry, School of Physical Sciences, University of Liverpool

[3] Centre for Preclinical Imaging, Institute of Systems, Molecular and Integrative Biology, University of Liverpool

Corresponding author's email: sgnmccal@Student.liverpool.ac.uk

INTRODUCTION

The clinical translation of cellular therapies has been limited due to a lack of convincing safety and efficacy data. Greater understanding of the mechanisms of action of cell therapies, as well as their fate and biodistribution, requires non-invasive imaging strategies(1). Hybrid PET/MRI imaging systems can offer superior cell tracking capabilities than their standalone counterparts(2). For this, cells must be labelled with imaging agents that can show a signal in both PET and MRI.

METHODS

Polymer coated Superparamagnetic-Iron-Oxide-Nanoparticles(SPIONs) were synthesised and labelled with the PET-tracer, Zirconium(Zr)-89. To assess colloidal stability, Zr-SIONs were incubated in cell culture medium (CCM). Particles were assessed for toxicity using an ATP assay, and their uptake into macrophages was assessed with Prussian Blue (PB) staining and a ferrozine assay.

RESULTS

Zr-SPIONs were found to aggregate in 10% and 0% FBS containing CCM. To improve colloidal stability, the polymer conformations of Zr-SPIONs were varied. Particles with a 1:4 ratio of DEAE-dextran to CM-dextran were colloidally stable in 10% FBS containing CCM. Zr-SPIONs did not affect the viability of the macrophages. From the PB staining assay, particles were found to take up efficiently into macrophages. A maximum intracellular iron content of 5 pg/cell was determined by the ferrozine assay.

CONCLUSIONS

Colloidally stable Zr-SPIONs, which showed minimal toxicity to macrophages and sufficient cellular uptake for contrast in PET/MRI imaging systems, were successfully synthesised. The next steps are to assess the biodistribution of Zr-SPION labelled macrophages in vivo in uninjured mice, and in an ischemia reperfusion model of kidney injury.

Keywords

SPION; PET/MRI; Cell tracking

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Abstract **#1347**

A NOVEL IMAGE-GUIDED MICROMECHANICAL EVALUATION METHOD TO STUDY CELL-MATRIX DEVELOPMENT IN TISSUE-ENGINEERED CONSTRUCTS

Manula SB Rathnayake$_P$[1], Manuela A Boos[1], Pholpat Durongbhan[1], Kathryn S Stok[1]

[1] *Department of Biomedical Engineering, The University of Melbourne*

Corresponding author's email: mboos@student.unimelb.edu.au

To develop functional cartilage tissue-engineered constructs, it is important to understand how they carry, distribute and respond to load as they develop. Current methods cannot investigate the role of tissue microenvironments on cell behaviour because they require direct manipulation of the cell to elicit a response. Thus, investigating how the load-bearing capacity of tissue components change as the tissue develops remains a challenge for engineering functional materials. Understanding the complex biomechanical behaviour of living tissues requires accurate measurement and visualisation of these systems in action.To address these questions an image-guided micromechanical evaluation approach is proposed using multiphoton microscopy coupled with mechanical compression of cartilage samples and tissue models in a custom-built device. To measure tissue strain under load, tissue volumes are acquired during the relaxation phases of a step stress-relaxation test. Using the open source digital volume correlation software, TomoWarp2 [1], displacement maps of the 3D structure are calculated. In this work, we present bovine auricular cartilage plugs (ø3-mm) with cell nuclei stained using SYTOX Green, and autofluorescence of fibrous components. The samples were imaged using multiphoton microscopy (Leica TCS SP8 MP) while mechanically compressed, and tissue displacement maps were calculated.The custom-device allowed for 3D imaging of cartilage samples under compressive load and, for the first time, a strain map of the cartilage tissue microenvironment at the individual matrix component level. In future this will be used to investigate the contributions of cells and ECM components to structural integrity, tissue development, and gain insight into tissue structure-function relationships.

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Abstract **#1348**

CHITOSAN-BASED ELECTRICAL CONDUCTIVE SCAFFOLDS FOR TISSUE ENGINEERING DEMANDS

Francesca Scalera[P][1], Anna Grazia Monteduro[1,2], Giuseppe Maruccio[1,2], Francesca Gervaso[1], Clara Piccirillo[1]

[1] *CNR NANOTEC Institute of Nanotechnology, via Monteroni, 73100 Lecce, Italy*

[2] *Department of Mathematics and Physics, University of Salento, via Monteroni, 73100 Lecce, Italy*

Corresponding author's email: francesca.scalera@nanotec.cnr.it

Electroconductive biomaterials, with promising effects on the regeneration of electrically active biological tissues such as neural, orthopaedic and cardiac tissues, have great potential in regenerative medicine applications. In this study, a chitosan-based composite scaffold has been developed by incorporating graphitic carbon obtained from cork (natural and sustainable source) as an electroconductive filler and subsequently characterized. Several concentrations of pyrolysed cork (PC) granules (0.01, 0.1, 0.5, 0.75, 1 wt%) were homogeneously dispersed into chitosan (Ch) slurry and freeze-dried. The prepared Ch-PC scaffolds were characterized in terms of electrical, mechanical, physicochemical and biological properties. An electroconductivity of 5.5x10-5 S/cm, i.e. in the range of cardiac tissues [1], was registered when a PC concentration of 1% wt was used. Though FTIR and XPS measurements showed no evidences of chemical bonds between the two components, the mechanical strength of composites scaffolds resulted significantly higher than pure Ch one, indicating that weak interactions arise between the two components, leading to an enhancement of the mechanical properties of the polymeric scaffolds. The biocompatibility of Ch-PC composite scaffolds has been verified by SH-SY5Y neuroblastoma cell viability assay. The ability of Ch-PC scaffold to support induced Pluripotent Stem Cells (iPSCs) differentiation into cardiomyocytes (iPSC-CMs) were also tested. In conclusion, this study shows that a composite made using an innovative electroconductive filler deriving from a natural source, such as PC, might have great perspectives in cardiac tissue engineering.

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Abstract **#1349**

INDUCTION OF A PATHOLOGICAL PHENOTYPE IN ARTICULAR CHONDROCYTE THROUGH INFLAMMATORY AND MECHANICAL STIMULI: A MULTISCALE IN SILICO APPROACH

Satanik Mukherjee[P][1,2], Raphaëlle Lesage[1,2], Liesbet Geris[1,2,3]

[1] *Prometheus, Division of Skeletal tissue Engineering, KU Leuven, Belgium*

[2] *Biomechanics Section, KU Leuven, Belgium*

[3] *GIGA In silico medicine, University of Liège, Belgium*

Corresponding author's email: satanik.mukherjee@kuleuven.be

Optimum mechanical signals are necessary to maintain the health of chondrocytes in articular cartilage. Abnormal mechanical signals can lead to departure from the stable chondrocyte homeostasis and a switch to hypertrophy, as observed in osteoarthritis(OA). For cartilage tissue engineering, the progenitor cells must be subjected to proper mechanical loading to stimulate chondrogenic differentiation and cartilage production. In this study, a coupled multiscale model of the human knee joint was developed to estimate the mechanical signals that the articular chondrocytes experience as a result of (patho)physiological loading at the joint-level. The intracellular processes triggered by said mechanical signals are modeled using a gene/protein regulatory network for mechanotransduction. Subsequently, changes in cell phenotype and activity of the relevant cell secreted factors are estimated. Using this multiscale model, the effect of meniscectomy on the progression of osteoarthritis is studied. Meniscectomy leads to increased strains in the articular cartilage as compared to a healthy joint, resulting in higher forces sensed by the chondrocytes and thereby initiating degenerative processes. With increased inflammatory cytokines in the meniscectomized joint, degeneration is increased further with the phenotypic switch of stable chondrocytes to hypertrophy when combined with mechanical loading experienced by the chondrocytes in the different zones of the cartilage. This multiscale model has huge potential in not only unraveling the role of mechanical loading in progression of OA but also delineating the role of mechanical loading in cartilage tissue engineering by quantifying the properties of osteochondral implants that will provide appropriate mechanical stimulation to the embedded progenitor cells.

Keywords

knee osteoarthritis; hypertrophic differentiation; gene/protein regulatory networks

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Abstract **#1350**

SCAFFOLD-FREE TISSUE-ENGINEERED FETAL CARTILAGE-DERIVED PROGENITOR CELLS PROMOTE MENISCUS REPAIR

Truong Minh-Dung$_P$[1], Park Do Young[2,1], Park In-Su[1], Choi Byung Hyune[3], Park So Ra[4], Min Byoung-Hyun[2,1,5]

[1] *Cell Therapy Center, Ajou University Hospital, Suwon, Korea*

[2] *Department of Orthopedic Surgery, Ajou University School of Medicine, Suwon, Korea*

[3] *Department of Biomedical Sciences, Inha University College of Medicine, Incheon, Korea*

[4] *Department of Physiology, College of Medicine, Inha University, Incheon, Korea*

[5] *Department of Molecular Science and Technology, Ajou University, Suwon, Korea*

Corresponding author's email: truongminhdung@gmail.com

This study aimed to determine whether meniscal tissue could be healed by the implantation of scaffold-free three-dimensional formed fetal cartilage-derived progenitor cells (FCPCs) (A-paste) in in vitro and in vivo evaluations. For in vitro, the characterization of A-paste cultured with/without fibrogenic induced medium resulted in increased meniscus formed features at 4 weeks. The in vitro remodeling process analysis of A-paste showed improved meniscus repair characteristics over time, with a higher integrated fibrocartilaginous matrix compared to meniscal autograft controls at 3 weeks and 6 weeks. The in vivo healing of meniscal defect was also confirmed at 6 weeks post-transplantation in a nude mouse model. In vivo investigation, twenty white New-Zealand rabbits (aged 20 weeks, 3.0-3.5 kg) were assigned to 2 groups. Defects 2 mm in diameter were created in the anterior horn of the medial meniscus. The defects were left empty in the control group and were filled with A-paste in the experimental group. Histological observations (Safranin-O stain, Masson Trichrome stain, and type 1 collagen) and meniscus repair scores were significantly better in the experimental group compared to the control group after 6 weeks and 12 weeks. In conclusion, our finding demonstrated A-paste has a fibrocartilaginous matrix formation and promotes meniscus healing in vitro, in vivo transplantation.

Abstract **#1351**

CHARACTERIZING THE EFFECTS OF ISTAROXIME EMBEDDED ELECTROSPUN SCAFFOLDS ON CARDIOMYOCYTES IN VITRO

Rabeil Sakina$_P$[1,2], Aida Llucià-Valldeperas[3], Sandro Gelsomino[4], Andrea Luraghi[5], Francesco Peri[5], Paul Wieringa[1], Lorenzo Moroni[1]

[1] Complex Tissue Regeneration Department, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University, Universiteitsingel 40, 6229 ER, The Netherlands

[2] Department of Biomedical Engineering and Sciences, School of Mechanical and Manufacturing Engineering, National University of Sciences and Technology (NUST), Islamabad, Pakistan

[3] Department of Pulmonology and Physiology, Vrije Universiteit University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam, the Netherlands

[4] Cardiothoracic Surgery Department, Maastricht University, Maastricht, the Netherlands

[5] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza, 2, 20126, Milan, Italy

Corresponding author's email: r.sakina@maastrichtuniversity.nl

Introduction

A drug-loaded cardiac patch can provide cues for stem cell maturation and function. Istaroxime is a novel therapeutic drug that enables upregulation of calcium signaling in cardiomyocytes to improve heart tissue function. Yet, istaroxime is severely limited by its short half-life of less than an hour (1). This renders the drug ineffective by the time it reaches the heart via the bloodstream. We therefore propose the combination of a drug-release mechanism with cardiomyocytes to treat heart tissue damage. The mechanism of drug embedding proposed here will therefore have the benefit of enabling sustained release of istaroxime from the biodegradable polymer.

Methods

Istaroxime was dissolved in hexafluoro-2-propanol (HFIP) and water (6ul/ml) to get 2% (w/v) istaroxime in 25% (w/v) Polyactive (300PEOT55PBT45) solution in solvents chloroform:HFIP (7:3). 25% Polyactive polymer solution without istaroxime was used as control. The scaffolds were electrospun and seeded with HL-1 cardiomyocytes. Cardiomyocytes were cultured on tissue culture plastic (TCP) and TCP supplemented with a daily dose of 100nM/100ul for 16 days. Cell proliferation was determined by metabolic activity and DNA assay. Live/dead imaging was used to assess cell viability over several time-points. Immunofluorescence staining was performed for several markers (SERCA2, actin and sarcomeric α-actinin). PCR analysis was carried out for cardiac specific genes.

Results

Metabolic activity showed increasing activity over time for all samples. DNA assay showed highest DNA content at day 16. Live/dead staining confirmed majority of live cells in culture over time. PCR analysis showed differences in several cardiac gene expression at day 8.

Keywords

istaroxime; electrospinning; cardiomyocytes

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Abstract **#1352**

INDUCED PLURIPOTENT STEM CELL-DERIVED VASCULAR NETWORKS TO SCREEN NANO-BIO INTERACTIONS

Luís Estronca$_P$[1], Vitor Franciso[2], Patricia Pitrez[2], Inês Honório[2], Lara Carvalho[3], Helena Vazão[2], Josephine Blersh[2], Akhilesh Rai[1], Xavier Nissan[4], Ulrich Simon[5], Mário Grãos[2], Leonor Saúde[3], Lino Ferreira[1]

[1] *Faculty of Medicine, University of Coimbra, 3000-548, Coimbra, Portugal*

[2] *Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*

[3] *Instituto de Medicina Molecular e Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal*

[4] *CECS, I-STEM, AFM, Institute for Stem Cell Therapy and Exploration of Monogenic diseases, Evry cedex, France*

[5] *Institute of Inorganic Chemistry, RWTH Aachen University, Germany*

Corresponding author's email: estronca@uc.pt

The use of nanomaterials-based technologies in biomedical applications poses challenges in terms of their vascular bioactivity/safety evaluation. Several methodologies have been proposed, however, they involve animal testing, are low-throughput, and they do not account for the biological differences between animals and humans. In this sense, the development of personalized human in vitro platforms to evaluate the interaction of nanomaterials with the vascular system would be important for therapeutic and regenerative medicine purposes. Here,we have used human induced pluripotent stem cells (hiPSCs)-derived endothelial cells (ECs) with “young” and “aged” phenotypes in 3 vascular network formats: 2D (polystyrene dishes), 3D (Matrigel) and a blood vessel-on-a-chip, to study their response to a library of 30 nanoparticles (NPs) used in imaging, antimicrobial and pharmaceutical applications, using, as a proof of concept, vascular toxicity as the main readout. The toxicity profile of NPs to hiPSC-ECs was dependent on the “age” of hiPSCs and the vascular network format. hiPSC-ECs were less susceptible to the cytotoxicity effect of NPs when cultured in flow than in static conditions, being the protective effect mediated, at least partially, by the glycocalyx. The results presented here highlight the relevance of in vitro hiPSC-derived vascular systems to screen vascular toxicity.

This work was funded by FEDER through the Program COMPETE and by Portuguese fund through FCT in context of the projects PTDC/SAU-TOX/121887/2010, POCI-01-0145-FEDER-029229, POCI-01-0145-FEDER-016390, POCI-01-0145-FEDER-029414,and by the European project ERAatUC (ref. 669088). The iMM Fish Facility was supported by CONGENTO LISBOA-01-0145-FEDER-022170, co-financed by FCT (Portugal) and Lisboa2020, under the PORTUGAL2020 agreement.

Abstract **#1354**

THE INFLUENCE OF DYNAMIC PHYSIOLOGICAL LOADING ON AN ENZYME-INDUCED EX VIVO MODEL OF INTERVERTEBRAL DISC DEGENERATION

Andrea Vernengo[P][1,2], Nadine Kluser[1,3], Astrid Soubrier[1], Jan Gewiess[1], Jan Ulrich Jansen[4], Cornelia Neidlinger-Wilke[4], Hans-Joachim Wilke[4], Zhen Li[1], Mauro Alini[1], Sibylle Grad[1]

[1] *AO Research Institute Davos*

[2] *Rowan University*

[3] *ETH Zurich*

[4] *Institute of Orthopaedic Research and Biomechanics Trauma Research Centre Ulm (ZTF)*

Corresponding author's email: andrea.vernengo@aofoundation.org

The long-term goal of this work is to develop a proteolytic enzyme digestion model of the intervertebral disc (IVD) for the study of regeneration therapies. In this study, we induce tissue digestion in a bovine organ culture model with two different enzymes, papain or chondroitinase ABC (ChABC), and assess the influence of dynamic loading on gene expression and disc dimensions after day 7 of culture. Papain and ChABC both induced glycosaminoglycan (GAG) loss and disorganization of tissue structure, but repetitive dynamic loading during enzymatic digestion induced significantly faster disc height loss compared to unloaded culture conditions ($p < 0.001$). ChABC was the only enzyme to induce statistically significant changes in gene expression compared to PBS injected controls. In the inner AF, mRNA expression relative to day 0 for ACAN and COL2 significantly increased ($p < 0.05$), whereas COL1 showed a trend towards downregulation. Loading did not induce any significant changes in gene expression in the inner AF. However, in the outer AF, loading induced a significant upregulation of COL1 ($p<0.05$) and downregulation of ACAN and COL2 ($p<0.05$). Currently, we are evaluating the cellular and molecular response of the IVD tissues over long term organ culture to understand if the observed patterns mimic that of human degeneration.

The work described is part of the iPSpine project, which has received funding from the European Union's Horizon 2020 Research and Innovation Programme (Grant No 825925).

Keywords

Enzyme; Organ culture; Degeneration model

Abstract **#1355**

3D MICROENVIRONMENTS TO ENGINEER BIOLOGICAL-INSPIRED VASCULARIZED BONE MODELS

Sofia Perea-Ruiz$_P$[1], Virginia Llopis-Hernández[1], K. H. Aaron Lau[2], Jian Ren Lu[3], Manuel Salmerón-Sánchez[1], Matthew J. Dalby[1]

[1] *Centre for the Cellular Microenvironment (CeMi), University of Glasgow, Glasgow, UK*

[2] *Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK*

[3] *Biological Physics Group, University of Manchester, Manchester, UK*

Corresponding author's email: sofiaperearuiz@gmail.com

One of the main challenges raised when engineering in vitro therapeutic systems is to combine multiples tissues to uncover the influence that other tissues have in the one under study. This interaction becomes essential since physiological responses are interconnected and they cannot be considered as isolated systems. In the case of bone metabolism, it has been shown that osteogenic events, angiogenesis and tissue repair are under neural control through direct interaction with neuropeptides [1],[2].

In the present work, a biological-inspired 3D vascularized bone model is introduced as a platform that presents vascular endothelial growth factor (VEGF) [25 ng/mL] and neuropeptide substance p (SP) [10-8M] tethered to poly-ethyl acrylate/fibronectin (PEA/FN) microbeads and embedded in a fibrin gel to induce vascularization. Human mesenchymal stem cells (hMSCs) seeded on the gel are close to the vascular system and the model is nanokicked to induce mechanically stimulated hMSCs osteogenesis. We hypothesized that SP may bind to FN, protecting it from degradation and creating a more stable presentation for the cells. Enzyme-linked immunosorbent assay, immunofluorescence and ellipsometry results show for the first time co-localization between both molecules, giving a thickness of 3 nm of SP bound on PEA/FN surfaces. On cellular studies with human vascular endothelial cells (HUVECs), results suggest that the combination of matrix-bound VEGF and SP synergistically enhances the sprouting, leading to a greater invasion of the gel after 7 days. In vitro evaluation of hMSCs also showed the highest osteogenic protein profile for osteopontin within the GF/peptide group after 14 days.

Keywords

Vascularisation; Neuropeptide; Growth factors

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Abstract **#1357**

DETERMINATION OF OPTIMUM PARAMETERS FOR SONICATION-ASSISTED DETERGENT PERFUSION IN THE PRODUCTION OF RENAL BIOSCAFFOLDS

Nathaniel Dugos[P1], Tosha Mae Manalastas[1], Gliceria Ramos[2], John Martin Mondragon[2], Neil Patrick Angeles[1], Custer Deocaris[3], Joseph Rey Sta. Agueda[1]

[1] *Chemical Engineering Department, De La Salle University, Manila, Philippines*

[2] *Biology Department, De La Salle University, Manila, Philippines*

[3] *Biomedical Research Section, Atomic Research Division, Philippine Nuclear Research Institute, Department of Science & Technology, Diliman, Quezon City, Philippines*

Corresponding author's email: nathaniel.dugos@dlsu.edu.ph

Detergent perfusion is the most common decellularization technique in producingextracellular matrix-derived renal bioscaffolds. However, it usually takes long treatmenttimes and large volumes of detergent solutions. Coupling this technique with sonicationtreatment has the potential to decrease the decellularization time but the combinedeffects of these two strategies can induce damage to the resulting bioscaffold. Thisstudy aims to determine the optimum decellularization parameters of sodium dodecylsulfate (SDS) concentration, flow rate, and sonicator power, which are parametersknown to have the most significant influence on the decellularization time andmicroarchitecture integrity of the scaffold, by using a response surface methodology.Cadaveric porcine kidneys were subjected to detergent perfusion and sonicationtreatment with varying SDS concentration, flow rate and sonication power.Decellularization time was recorded as the time it took for the kidney to transition into awhite translucent color upon SDS perfusion while the microarchitecture integrity scorewas done through a scoring system based on the H&E stained structures of theglomerulus, tubules, and blood vessels. Results show that using the optimumdecellularization parameters of 0.71% wt/vol SDS, 45 mL/min flow rate, and 60 Wsonication power, an acellular ECM scaffold was obtained after 3 hours with onlyminimal to moderate disruption of the renal structure. Colorimetric assays andimmunohistochemistry tests showed the retention of ECM proteins such as total solublecollagen, fibronectin, and laminin. The optimum parameters of sonication-assistedperfusion result in the efficient production of acellular renal bioscaffolds with preservedstructural integrity.

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Abstract **#1358**

PERFUSION BIOREACTOR FOR THE STUDY OF THE EPICARDIUM IN 3D ORGANOTYPIC CULTURE

Paola Campagnolo$_P$[1], Davide Maselli[1], Rolando Matos[1], Robert Johnson[1], Ciro Chiappini[2], Patrizia Camelliti[1]

[1] *University of Surrey*

[2] *Kings College London*

Corresponding author's email: p.campagnolo@surrey.ac.uk

Introduction: Following cardiac injury, the adult epicardium becomes a source of a multipotent cardiac progenitors and paracrine signals contributing to the repair process(1) and therefore represents a unique target to improve post-ischemic recovery. We developed a robust ex-vivo 3D organotypic model enabling the study of the epicardium and the development of strategies to enhance its potential.

Methods: Thin epicardial/cardiac slices (EpCardio-TS) are obtained from porcine hearts and cultured for up to 72h in a bioreactor system based on a 3D printed chamber connected to a control system ensuring stable and tuneable culture conditions and maintaining the air-liquid interface. Epicardial cell tracking is performed using localised nanoinjection(2) of fluorescent dyes or by immunostaining. 3D slices morphology is visualised by immunofluorescence on decolourised slices.

Results: EpCardio-TS presents both a healthy epicardium and an electrically connected myocardium. The optimized culture conditions preserve the viability of the epicardium upon culture as indicated by calcein staining (T0: 68.0±3.9%; 24h: 60.2±8.2% 48h 72.9±8.5%) and apoptosis quantification (T0: 7.42±1.69%; 24h: 24.9±6.6%; 48h: 25.5±7.2%). Treatment with thymosin β4 (Tβ4) reactivates the epicardial cells increasing the expression of embryonic and epithelial-to-mesenchymal transition genes and resulting in improved motility into the myocardium and mesenchymal differentiation.

Discussion&Conclusions: Our 3D organotypic culture uniquely enables drug and gene therapy screening to boost the epicardial contribution to heart repair and remodelling. Our data from porcine heart tissue are physiologically relevant to the human heart, confirming the prominent role of the epicardial cells in the myocardial repair in this patient-relevant model.

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Abstract **#1359**

THERMO-SENSITIVE CHITOSAN-BASED HYDROGELS FOR NEURAL AND MUSCLE CELL ENCAPSULATION: TOWARD A 3D NEURO-MUSCULAR JUNCTION IN VITRO

Antonella Stanzione$_P$[1,2], Alessandro Polini[3], Alessandro Romano[4], Velia La Pesa[4], Pamela Mozetic[3], Lorenzo Moroni[3,5], Giuseppe Gigli[3,1], Francesca Gervaso[3]

[1] *Università Del Salento, Dipartimento di Matematica e Fisica E. de Giorgi, Campus Ecotekne, via Monteroni, Lecce, 73100, Italy*

[2] *1 CNR NANOTEC – Institute of Nanotechnology, Campus Ecotekne, via Monteroni, Lecce, 73100, Italy*

[3] *CNR NANOTEC – Institute of Nanotechnology, Campus Ecotekne, via Monteroni, Lecce, 73100, Italy*

[4] *IRCCS San Raffaele Scientific Institute, Division of Neuroscience, Institute of Experimental Neurology, Milan, 20132, Italy*

[5] *Maastricht University, Complex Tissue Regeneration, Universiteitssingel 40, Maastricht, 6229 ER, Netherlands*

Corresponding author's email: antonellastanzione00@gmail.com

Extracellular microenvironment and cell-ECM crosstalk strongly influence cellular behavior through their mutual exchange of bio-chemical and physical signals [1-2]. Several in vitro studies showed the importance of a three-dimensional (3D) microenvironment architecture in many cellular processes, such as migration and differentiation [3-4]. 3D culture systems have been successfully proposed to construct analogous tissues for tissue engineering and regenerative medicine applications and, in the last decade, as disease models, useful for evaluating drug effects in vitro [5-6]. In this perspective, hydrogels are valid biomaterial candidates that can be used as 3D culture systems, featuring highly tunable physical-chemical and biological properties. Aiming to recapitulate in vitro the Neuro-Muscular Junction (NMJ), we propose chitosan-based thermo-sensitive hydrogels capable of gelling at 37°C with the addition of organic and inorganic saline solutions (beta-glycerol phosphate and sodium hydrogen carbonate, respectively). The developed formulations have physico-chemical properties suitable for cell encapsulation, show long term stability (up to three weeks) and tunable mechanical properties (in the range 6-10 KPa) and permeability. Biological analyses revealed that the proposed hydrogels are biocompatible, showing a marked viability (>90%) and metabolic activity of both neural (NSC34) and muscle (C2C12) murine cells. Finally, the hydrogels demonstrated to be able to support neuronal (neurofilament-H) and muscle cell (Myo-D, myogenin, myosin heavy chain) differentiation.

Keywords

3D cell culture; Neuromuscolar juction; Responsive-hydrogel

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Abstract **#1361**

BIOENGINEERED TRANSMURAL CARDIAC CONSTRUCTS WITH HEXAGONAL MICROARCHITECTURAL RESOLUTION

Nino Chirico[P][1,2], Madison Ainsworth[1,3], Jan Willem Buikema[1,2], Inge Dokter[1,2,3], Renee Maas[1,2], Joos Malda[1,3,4], Miguel Castilho[1,3,5], Alain van Mil[1,2], Joost Sluijter[1,2]

[1] Regenerative Medicine Center Utrecht, Circulatory Health Laboratory, University Utrecht, University Medical Center Utrecht, Utrecht, the Netherlands

[2] Laboratory of Experimental Cardiology, Department of Cardiology, University Medical Center Utrecht, Utrecht, the Netherlands

[3] Department of Orthopaedics, University Medical Center Utrecht, Utrecht, the Netherlands

[4] Department of Equine Sciences, Faculty of Veterinary Sciences, Utrecht University, Utrecht, the Netherlands

[5] Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands

Corresponding author's email: n.chirico@umcutrecht.nl

Current in vitro 3D cardiac tissue models do not fully replicate mature myocardial function or micro-architecture. Previously, we fabricated melt electrowritten (MEW) hexagonal microfiber meshes with biomechanical properties that mimic native myocardium, thereby supporting human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) contraction and driving tissue organization and maturation[1]. We hypothesized that incorporating cardiac fibroblasts and providing contact guidance and reinforcement through strategically stacked MEW meshes, enables the replication of transmural 3D macrostructure and function. In this study, we present the generation of a bioengineered transmural cardiac construct (TmC).

Hexagonal MEW meshes were fabricated using a 3DDiscovery (RegenHU). TmCs of 8mm in diameter and 10mm in thickness were produced by stacking multiple MEW meshes, seeded with gelMA-collagen hydrogels encapsulating hiPS-CMs and hiPS cardiac fibroblasts (hiPS-cFBs)[2] (9:1), and cultured for four weeks. Function, morphology, and maturation were evaluated using construct contraction analysis, histology, and cardiac marker expression (immunofluorescent staining and qRT-PCR).

Beating cell clusters were observed 3-4 days after TmC seeding, proceeded by whole construct synchronized beating (40bpm) after 10-14 days, including construct radial deformation of 1-2mm. Histological sections exhibited a clear multi-layered structure with a uniform cellular distribution. Immunofluorescent staining showed hiPS-CMs and hiPS-cFBs organized into tissue-like structures, aligned with the hexagonal microarchitectures. An increased expression of cardiomyocyte maturation-related genes (RYR2/TNNI3/CPT1B) was observed in TmCs compared to single-mesh constructs.

Our study provides an important step towards the generation of 3D in vitro cardiac models of relevant dimensions with native-like tissue architecture and function.

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Abstract **#1362**

CYCLIC STRETCH TRIGGERS AN ALTERED CELLULAR ACTIVITY IN A FIBROTIC MICROENVIRONMENT

Linda Elowsson Rendin[P][1], Anna Löfdahl[1], Bryan Falcones[2], Isaac Almendros[2], Jorge Otero[2], Xiao-Hong Zhou[3], Göran Dellgren[4], Martin Silverborn[4], Ramon Farré[2], Gunilla Westergren-Thorsson[1]

[1] *Lung Biology unit, Lund University*

[2] *Unit of Biophysics and Bioengineering, Universitat de Barcelona*

[3] *AstraZeneca*

[4] *Sahlgrenska University Hospital*

Corresponding author's email: linda.elowsson_rendin@med.lu.se

Chronic lung diseases such as idiopathic pulmonary fibrosis (IPF) involves extensive remodeling of the extracellular matrix (ECM), primarily in the periphery of the lung. This leads to altered mechanical properties, which in turn results in altered mechanical stimulation of the cells. In stiffer and less elastic ECM found in IPF, fibroblasts are thus exposed to altered strain compared to a healthy ECM. Our aim is to investigate the impact of mechanical stimulation on fibroblast activity in IPF. Decellularized lung slices (350 um thickness) were attached to a custom-made device for cyclic stretch built in polydimethylsiloxane and repopulated with healthy primary distal lung fibroblasts. The lung scaffolds were either exposed to cyclic stretch (0.2 Hz, 10% strain) or cultured under static conditions upto 3 days and analyzed for cell viability, histology, RNA expression, and released mediators. Compared to healthy scaffolds, fibroblasts on IPF scaffolds showed increased metabolic activity, both at static and cyclic stretch condition. Scaffolds under cyclic stretch showed preserved tissue morphology with dispersed elongated fibroblasts shown with H&E staining. Interestingly, we see a shift in released mediators linked to angiogenesis in cyclic stretch cultures, mostly pronounced in IPF scaffolds. We demonstrate that we have developed a physiological more relevant ex vivo model to study cellular responses in lung tissue. Our data indicate that there is a change in cellular activity in IPF tissue under cyclic stretch. This allows us to study the effect of alterations in mechanical and matrisome properties in chronic lung disease.

Abstract **#1363**

MACROPHAGE POLARIZATION AND IN VITRO INNATE AND ADAPTIVE IMMUNE RESPONSE OF NATIVE AND REGENERATED SILK-BASED BIOMATERIAL

Sourabh Ghosh[P][1], Subhadeep Roy[1], Aarushi Sharma[1]

[1] *Regenerative Engineering Laboratory, Department of Textile Technology, Indian Institute of Technology, New Delhi 110016, India*

Corresponding author's email: sourabh.ghosh@textile.iitd.ac.in

Biomaterials should be characterized by a controlled capacity to activate the immune system promoting wound healing and regeneration process, while avoiding chronic inflammation and "foreign body" responses. Majority of studies on silk fibroin have naively claimed this polymer scaffold/hydrogel material as 'biocompatible' or 'biologically safe'. This term 'biocompatible' should be used with caution, as this claim indicates that silk fibroin-based biomaterials can augment tissue regeneration, smooth integration with host tissue and trigger biological functionality without causing chronic inflammation and foreign body response1. Physical characterization (FTIR, AFM, XRD) of Silk fibroin Bombyx mori (BM) and Antheraea mylitta (AM) 3D silk textile braids were compared with 2D BM film (β-crystallized using water-annealed and ethanol treatment). Monocyte cell line THP-1 and T cells isolated from healthy donors were cultured over those scaffolds. We have selected a specific panel of cytokines microarray, cell surface markers, Nitric oxide estimation, cellular morphological changes that will help to elucidate the different stages of macrophage polarization and T cell activation on silk biomaterial surface in a synchronize pattern. This comprehensive study will largely help to elucidate the basic paradigm of immune response in context with surface topography, 3D structure, matrix stiffness, processing parameters, conformational changes of different form of silk-based biomaterial and provide future direction to build proper immune instructive silk-based biomaterials for tissue regeneration.

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Abstract **#1364**

MICROFABRICATED SYSTEMS FOR THE STUDY OF NEURODEGENERATIVE DISORDERS

Eleonora De Vitis$_P$[1,2], Velia La Pesa[3], Francesca Gervaso[1], Alessandro Romano[3], Angelo Quattrini[3], Giuseppe Gigli[2,1], Lorenzo Moroni[4], Alessandro Polini[1]

[1] *CNR Nanotec - Institute of Nanotechnology*

[2] *Università del Salento*

[3] *IRCCS San Raffaele Scientific Institute*

[4] *Maastricht University*

Corresponding author's email: eleonora.devitis@nanotec.cnr.it

Understanding the complex communication between different cell populations and their interaction with the microenvironment in the central and peripheral nervous systems is fundamental in neuroscience research. Due to the lack of suitable animal models capable of faithfully reproducing the physio-pathological mechanisms of many human diseases, the development of appropriate in vitro approaches and tools, able to selectively analyze and probe specific cells and cell portions (e.g., axons and cell bodies in neurons) has become therefore crucial in this direction. Here, a protocol was established to microfabricate a microfluidic chip by multi-level optical lithography. We evaluated different microfluidic setups and coating materials, as well as several geometric features: three perfusable cell compartments (500 µm wide and 6 mm long) with distinct inlets and outlets were interconnected through a series of narrow and parallel microgrooves (either 2.5, 5 or 10 µm wide and 250 µm long). We showed that an accurate choice of device geometrical features and cell culture parameters (i.e., cell density and coating procedures) allowed to: i) maximize cell adhesion and proliferation of neuron-like human cells (SH-SY5Y cells) and primary rat Schwann cells, ii) perform on-chip cell differentiation towards more physiologically relevant phenotypes (i.e., differentiated mature adherent neurons and mature myelinating Schwann cells), ii) control the inter-compartment cell migration, iii) execute long-term cell culture studies. These results can lead to a plethora of in vitro co-culture studies in the neuroscience research field, where tuning and investigating cell-cell and cell-microenvironment interactions are essential.

Abstract **#1365**

EASY HANDLING AND INJECTABLE CEMENT FOAM FOR BONE TISSUE ENGINEERING

Moussi Hilel[P][1,2], Veziers Joëlle[1], Rouillon Thierry[1], Gauthier Olivier[1], Schaefer Aurélie[1], Billon-Chabaud Aurélie[3], Tancret Franck[2], Quillard Sophie[2], Terrisse Hélène[2], Geoffroy Valérie[1], Weiss Pierre[1], Le Bideau Jean[2], Gauthier Hélène[3]

[1] *Université de Nantes, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France*

[2] *Institut des Matériaux Jean Rouxel, Université de Nantes, CNRS UMR 6502, Nantes, France*

[3] *Faculté de Pharmacie, Université de Nantes, Nantes, France*

Corresponding author's email: hilel.moussi@etu.univ-nantes.fr

Calcium phosphate cements (CPC) are widely used as bone substitutes because of their similar composition with the mineral phase of bone and of their injectability, making them of particular interest in mini-invasive surgery. However, their bone healing properties are not sufficient to promote osteogenesis due to a poor macro-porosity or an inadequate resorbability preventing cell migration and vascularization. In order to combine interconnected macro-porosity and injectability, we perfected a promising approach, using a dual syringe mixing technique1, to produce foams composed of CPC and polymer with foaming agent. Among a dozen of formulations containing calcium deficient hydroxyapatite cement (CDHA) associated with different hydrogels were tested (e.g., alginate, chitosan, hyaluronic acid, gelatin). We selected two of them based on their biocompatibility and ability to form a hydrogel foam: 1) a silanized hyaluronic acid hydrogel creating the three-dimensional network and Albumin as foaming agent (Si-Hya Ac + RSA); 2) a foaming gelatin-based hydrogel (Gel). Their macro-porosity between 100 and 300 µm and their young's modulus of 0.5 GPa for CDHA/Si-Hya Ac + RSA and 1.8 GPa for CDHA/Gel respectively were closed to the porous ceramics2. Moreover, CDHA/Si-Hya Ac + RSA and CDHA/Gel on STRO1 cells allowed to maintain their viability over 3 days. In vivo, critical size defects (6x10mm, n=6/condition) were performed in femoral metaphysis of New-Zealand white rabbits. Defects filled with the two new biomaterials exhibited greater mineral formation compared to the control, one week after the surgery suggesting a bone regeneration potential of our new biomaterials.

Keywords

calcium phosphate cement ; hydrogel; bone repair

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Abstract **#1366**

DEVELOPMENT OF APPROACHES FOR A TWO-LAYER PATCH COMBINING ELECTROSPINNING AND 3D PRINTING WITH IMPROVED ELECTRICAL STIMULUS TRANSMISSION AND SELF-REGULATING VISCOELASTIC PROPERTIES FOR APPLICATION IN CARDIAC TISSUE ENGINEERING

Aldo Boccaccini$_P$[1], Lena Vogt[1], Florian Ruther[1], Kaveh Roshanbinfar[1], Felix Engel[2]

[1] *University of Erlangen-Nuremberg*

[2] *Univ. of Erlangen-Nuremberg*

Corresponding author's email: aldo.boccaccini@ww.uni-erlangen.de

Cardiovascular diseases like myocardial infarction are still significant causes of morbidity and mortality worldwide1. The limited intrinsic self-regeneration ability of cardiac muscle tissue after damage by myocardial infarction, as well as restricted therapies and the lack of donor organs demand new strategies. Among those, the cardiac patch strategy represents an important approach, which can not only provide mechanical support to the infarcted area but also be a platform for healthy cell delivery2. To achieve a highly functional cardiac patch, collagen, hyaluronic acid, and polyaniline (PANi) were electrospun to form the top layer of a bilayered patch3. Homogeneous and defect-free fiber mats with fiber diameter in a range between 120 and 350 nm were obtained. Increasing PANi concentration led to stronger, but less elastic fiber mats. Electrical conductivities close to the human myocardium with up to 2.0 ± 0.6 mS/cm were achieved. Although the cardiomyocyte viability was slightly decreased with the addition of PANi, the beating rates of cardiomyocytes did not show significant differences for the different compositions. The highest PANi composition revealed a significant increased contraction amplitude and connexin43. This physico-chemically mimetic fiber mat could now be combined with a highly patterned scaffold. Thus, a combination of the polyester poly (glycerol sebacate) (PGS) and the maiz protein zein was 3D printed using an extrusion-based system to achieve sufficient mechanical properties close to native myocardium. PGS-Zein obtained good printability with little shrinkage during heat crosslinking. Cyclic fatigue tests indicated no significant material damage to the printed structure after 5000 cycles.

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Abstract **#1367**

TAILORING OF FLOW PROPERTIES OF GELATIN METHACRYLOYL VIA UV PHOTOCROSSLINKING TO YIELD EXTRUDABLE HYDROGELS FOR 3D BIOPRINTING

Paulo Alexandre Amorim[1]

[1] *KU Leuven*

Corresponding author's email: pauloalexandre.amorim@kuleuven.be

Gelatin methacryloyl (gelMA) hydrogels are widely used biomaterials in tissue engineering, such as bioprinting [1]. However, due to its highly predominant elastic behavior, the 3D bioprinting of this material via extrusion results in poor surface aspect and mechanical integrity[2], which is probably associated with their low ability to flow, culminating in brittle fracture. Hereby, we report the obtention of a gelMA chemical hydrogel capable of transitioning from solid to liquid-like behavior under stress, enabling the conception of inks for extrusion-based bioprinting. Samples of gelMA hydrogels with high degree of substitution (95%) at the concentration of 10 w/v% were photocrosslinked in presence of a photoinitiator, lithium phenyl-2,4,6-trimethyl benzoyl phosphinate (Li-TPO-L), at the concentrations of 0.1 and 0.5 w/v% for 10 min at 365 nm. To assess the chemical hydrogels ability to flow and the presence of gel state, strain amplitude and frequency sweep tests were performed at 37 °C using a Anton Paar Physica MCR 501 rheometer, 25 mm paralallel plates and solvent trap. The physical hydrogel was tested under the same setup at 20 °C. Strain-amplitude tests showed that the formulation containing 0.1 w/v% of Li-TPO-L, photocrosslinked for 10 min presented transition from solid to liquid-like behavior whereas the GelMA/0.5 w/v% Li-TPO-L and the physical hydrogel remained as viscoelastic solids. Frequency sweep tests showed that all the materials behave as gels throughout the applied frequency range, which is key for the development of bioinks. Therefore, this strategy can be useful for obtaining constructs of superior integrity.

Keywords

gelMA; rheology; extrusion-based bioprinting

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Abstract **#1368**

TISSUE ENGINEERING LONG-TERM HUMAN SKIN MODELS

Rahul Rimal[P][1], Yvonne Marquardt[2], Suzana Djeljaidni[1], Thomas Neovalis[1], Dmitry Chigrin[1], Matthias Wessling[1], Jens Malte Baron[2], Martin Möller[1,3], Smriti Singh[1]

[1] *DWI-Leibniz Institute for Interactive Materials, Forkenbeckstrasse 50, 52074 Aachen, Germany*

[2] *Department of Dermatology and Allergology, University Hospital, RWTH Aachen University, 52074 Aachen, Germany*

[3] *A.N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Science, Vavilova 28, 119991 Moscow, Russia*

Corresponding author's email: rimal@dwi.rwth-aachen.de

There is a growing need of in-vitro human skin models for skin disease modelling and drug testing. To mimic native skin, conventional models utilize scaffolds such as collagen. However, scaffold-based methods undergo tissue contraction that prohibits it´s use for long-term study. Here, we fabricated scaffold-free skin models to eliminate such limitations. The skin models were engineered using the cell-coating and accumulation technique where human fibroblasts were coated layer-by-layer with extracellular moieties and accumulated in cell culture inserts. Keratinocytes were seeded on top of the scaffold-free dermis and the full-thickness skin model was cultivated in air-liquid interface. This scaffold-free technique resulted in highly durable non-constricting skin constructs. Owing to this stability, the skin models were utilized to initiate psoriasis skin disease, test anti-psoriatic drug for a time-frame of 14 days and observe the influence of the drug on psoriasis phenotype. Another goal of the study was to incorporate endothelial cells and fabricate a long-term vascularized skin models. Vascularization, however, led to several challenges such as degradation of the dermal compartment and abnormal vessel growth due to the interplay between keratinocytes and vessels. This hindered the stability of the fabricated vascularized skin tissue. Keratinocytes were observed to influence vessel architecture by releasing excess growth factors such as the vascular endothelial growth factors (VEGF). To combat these challenges, engineered skin models were provided with a dynamic environment which prevented the dermal degradation and vessel abnormality. Together, in this study, we engineered a long-term and durable vascularized and non-vascularized skin models.

Abstract **#1369**

TGF-BETA INDUCES RIBOSOME ACTIVITY, ALTERS RIBOSOME COMPOSITION AND INHIBITS CITE-MEDIATED TRANSLATION

Guus G.H. van den Akker$_P$[1], Alzbeta Chabronova[1], BAS A.C. Housmans[1], Andy Cremers[1], Don A.M. Surtel[1], Marjolein M.J. Caron[1], Mandy Peffers[2], Tim J.M. Welting[1]

[1] *Maastricht University Medical Center+, department of Orthopedic Surgery*

[2] *University of Liverpool, Institute for Ageing and Chronic disease*

Corresponding author's email: g.vandenakker@maastrichtuniversity.nl

Ribosomes are required for the continuous translation of all proteins in cells. Recently, it was discovered that thousands of human mRNAs contain a 5' untranslated region that regulates protein translation through Cap independent translation elements (CITE). We used a candidate approach in a chondrocytic cell line to identify growth factors or cytokines that differentially regulate CITE-mediated translation.

Our screening revealed that TGFβ3 consistently decreased CITE activity in favor of Cap-mediated translation. A concentration of ≥ 1 ng/ml and at least two days of stimulation was required for this effect. Mass-spectrometry with label-free quantification was used for proteomic analysis. In the cellular proteome we found that TGFβ3 induced protein expression of known TGFβ transcriptional target genes compared to control. This was also reflected in the secretome. Finally, we isolated ribosomes with their associated proteins from control and TGFβ3-treated cells. Ribosomes isolated from TGFβ3-treated cells contained significantly lower amounts of HNRNP family members. The majority of these HNRNPs were not differentially expressed in the cellular proteome and are known to be involved in CITE translation. Finally, a small but significant increase of specific ribosomal proteins was found in ribosomes extracted from TGFβ3- treated cells.

We demonstrate that a growth factor/cytokine can induce ribosome activity, alter ribosome composition and modulate the preferential mode of translation in eukaryotic cells. Future experiments will focus on functional validation of the link between ribosome-associated HNRNP abundance and alterations in CITE-mediated translation. Ribosomes might present a drugable target in complex diseases where TGFb plays a role.

Abstract **#1370**

THE EFFECT OF DIAMETER OF FIBRE ON FORMATION OF HYDROGEN BONDS AND MECHANICAL PROPERTIES OF 3D-PRINTED PCL

Żaneta Góreckaₚ[1], Joanna Idaszek[1], Dorota Kołbuk[2], Emilia Choińska[1], Adrian Chlanda[3], Wojciech Święszkowski[1]

[1] Warsaw University of Technology, Faculty of Material Science and Engineering, Wołoska 141, 02-507 Warsaw, Poland

[2] Polish Academy of Sciences, Institute of Fundamental Technological Research, Pawińskiego 5b, 02-106 Warsaw, Poland

[3] Institute of Electronic Materials Technology, Department of Chemical Synthesis and Flake Graphene, Wólczyńska 133, 01-919 Warsaw, Poland

Corresponding author's email: zaneta.gorecka.dokt@pw.edu.pl

Fused Deposition Modelling (FDM) technique has been widely utilized in fabrication of 3D porous scaffolds for tissue engineering (TE) applications. Surprisingly, although there are many publications devoted to the architectural features of the 3D scaffolds fabricated by the FDM, none of them give us evident information about the impact of the diameter of the fibres on material properties. Therefore, the aim of this study was to investigate the effect of the diameter of 3D-printed PCL fibres on variations in their microstructure and resulting mechanical behaviour. The fibres made of poly(ε-caprolactone) (PCL) were extruded through commonly used types of nozzles (inner diameter ranging from 0.18 mm to 1.07 mm) by means of FDM technique. Static tensile test and atomic force microscopy working in force spectroscopy mode revealed strong decrease in the Young's modulus and yield strength with increasing fibre diameter in the investigated range. To explain this phenomenon, we conducted DSC, WAXS, FTIR, IR and PLM imaging. The obtained results clearly showed that the most prominent effect on the obtained microstructures and mechanical properties had different cooling and shear rates during fabrication process causing changes in supramolecular interactions of PCL. The observed fibre size-dependent formation of hydrogen bonds affected the crystalline structure and its stability. Summarising, this study clearly demonstrates that the diameter of 3D-printed fibres has a strong effect on obtained microstructure and mechanical properties. Moreover, results suggest the impact on cellular response.

This study was supported by the National Centre for Research and Development in Poland (POIG.01.01.02-00-022/09; STRATEGMED1/233624/4/NCBR/2014; STRATEGMED3/306888/3/NCBR/2017).

Keywords

policaprolactone; hydrogen bonds; 3D printing

Abstract **#1371**

CREATING MULTI-FUNCTIONAL FIBROUS CELL-SCAFFOLD INTERFACES BY UTILIZING VERSATILE NCO-SP(EO-STAT-PO) PRE-POLYMER

Mehmet Berat Taskin$_P$[1], Tina Tylek[1], Carina Blum[1], Thorsten Keller[1], Christoph Böhm[1], Jürgen Groll[1]
[1] *University Hospial Würzburg - Department for Functional Materials in Medicine and Dentistry*
Corresponding author's email: mehmet.taskin@fmz.uni-wuerzburg.de

Functional polymers play a significant role in the development of novel biomaterials for various applications. Among them six-arm star-shaped poly(ethylene oxide-stat-propylene oxide) with NCO terminal groups (NCO-sP(EO-stat-PO)) had demonstrated a great potential. Apart from its use as a functional surface coating, NCO-sP(EO-stat-PO is embedded within fibrous polymer networks that are fabricated via electrospinning. As a versatile cytocompatible pre-polymer, NCO-sP(EO-stat-PO can be used to stabilize fibrous gelatin network in aqueous conditions, and negates the use of possibly toxic additional crosslinking agents. Its star shaped functional arms form into a dense crosslinked network which creates a highly hydrophilic shell. Hence, when blended with synthetic hydrophobic polymers such as PCL and PLGA and spun into fibrous meshes, it increases the hydrophilicity of scaffolds and minimizes the non-specific protein adsorption. This bioinert surfaces with low protein adsorption inhibit adhesion of e.g. fibroblasts, hMSCs. In contrast, such interfaces help to mitigate excess anti-inflammatory responses caused by macrophages. Scaffolds can be further specifically bioactivated with cell instructive cues e.g. adhesion peptides, via -NCO terminal groups on star-shaped polymer. Overall, the possibility of multi-modal bioactivation, tuning the density of NCO-sP(EO-stat-PO on the surface of fibers together with the relevant bioactive molecules manifest a tremendous capacity for the fabrication of cell-instructive scaffolds.

Keywords
Anti-inflammation; Macrophage polarization; stem cell adhesion - differentiation

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Abstract **#1372**

INVESTIGATION OF THE MECHANICAL PROPERTIES OF CELL/HYDROGEL CONSTRUCTS FOR BIOPRINTING OF CARDIOVASCULAR IMPLANTS

Shannon Anna Jung[P][1,2], Miriam Aischa Al Enezy-Ulbrich[1,2], Svenja Wein[3,4], Christian Böhm[5], Norina Labude-Weber[3,4], Hanna Malyaran[3,4], Stefan Jockenhoevel[5,6], Sabine Neuss[3,4], Andrij Pich[1,2]

[1] Institute for Technical and Macromolecular Chemistry, Research Area Functional and Interactive Polymers, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[2] DWI - Leibniz Institute for Interactive Materials, RWTH Aachen University, Forckenbeckstraße 50, 52074 Aachen, Germany

[3] Helmholtz Institute for Biomedical Engineering, BioInterface Group, RWTH Aachen University, Pauwelsstrasse 20, 52074 Aachen, Germany

[4] Institute of Pathology, RWTH Aachen University, Pauwelsstrasse 30, 52074 Aachen, Germany

[5] AME BioTex – Biohybrid & Medical Textiles, RWTH Aachen University, Forckenbeckstraße 55, 52070 Aachen, Germany

[6] Aachen-Maastricht Institute for Biobased, Sciences, Faculty of Science and Engineering, Maastricht, Netherlands

Corresponding author's email: shannon.jung@rwth-aachen.de

As a result of global rise in ageing populations, heart valves diseases have become a major health issue world-wide. The most widely used treatment for heart valve diseases are surgical replacement strategies with mechanical or biological heart valves implants. However, there are large drawbacks including the formation of blood clots (mechanical implants), short durability and limited growing, repair and remodel ability. In the current work, researchers focus on tissue engineered heart valves supporting both biocompatibility and reproducibility of implants.

Here, three-dimensional cell/hydrogel constructs are synthesized for the application in biohybrid heart valves. An investigation of the mechanical properties of fibrin-based hydrogels with L929 cells is conducted and a bioprinting process of the cell/hydrogel construct is explored. The gels are synthesized from natural fibrin and synthetic linear reactive copolymers based on poly(N vinylpyrrolidone) and can provide a three-dimensional environment for cell proliferation and growth. Due to cost and easier handling of cell lines compared to patient-derived, primary cells, L929 cells are used for initial experiments to determine the influence of the cells on the mechanical properties of the hydrogels and for the optimization of the bioprinting process. Rheological experiments proof that the cell concentration has no significant influence on the hydrogels mechanical properties. Furthermore, the possibilities to fabricate cell/hydrogel constructs with different forms (e.g. microbeads or films) will be explored using extrusion based bioprinter. In future experiments we will focus on the development of the printing process of cell/hydrogel constructs and the analysis of the cell viability after the printing process.

Abstract **#1373**

DEVELOPMENT AND CHARACTERIZATION OF ADIPOSE-DERIVED STEM CELL-BASED SELF-ASSEMBLED SCAFFOLD FOR FUTURE URETHRAL TISSUE ENGINEERING

Zahra Rashidbenam[P][1], Mohd Hafidzul Jasman[2], Xeng Inn Fam[2], Christopher Chee Kong Ho[3], Zulkifli Md Zainuddin[2], Reynu Rajan[2], Fatimah Mohd Nor[2], Mohamad Aznan Shuhaili[2], Nik Ritza Kosai[2], Farrah Hani Imran[2], Min Hwei Ng[1]

[1] Centre for Tissue Engineering and Regenerative Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia

[2] Department of Surgery, Universiti Kebangsaan Malaysia Medical Centre, Clinical Block, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000 Kuala Lumpur, Malaysia

[3] School of Medicine, Taylor's University, No. 1 Jalan Taylor's, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia

Corresponding author's email: Zahra_Rashidbenam@yahoo.com

The urethral stricture, which develops due to scarring process, is treated by urethral dilation and urethrotomy; but in complex and long urethral strictures, substitution urethroplasty is the only option. Autologous genital skin and buccal mucosa grafts are currently used in urethral reconstruction; however risk of complications such as hair ingrowth in the urethra, stone formation and donor site morbidity, restrict the application of these grafts. To overcome this complication, a full thickness, human urethra consisting urothelium and smooth muscle layers on a self-assembled scaffold derived from adipose derived stem cells (ASCs) had been constructed. To obtain a thick self-assembled scaffold, the effect of cell seeding density, O2 level concentration and ascorbic acid concentration were examined under static and dynamic culture conditions. Findings showed that reducing the oxygen level, increasing the ascorbic acid concentration and mechanical stimulation during ASCs culture increases the cell sheet thickness. The biocompatibility assessment of ASC-based self-assembled scaffold showed that seeded urothelial cells (UCs) and smooth muscle cells (SMCs) on the cell sheet were able to grow and proliferate for up to 14 days. Immunocytochemistry analysis revealed that ASC-based self-assembled scaffold was able to maintain the UCs markers (Ck7, CK20, UPIa and UPII) and SMC markers (α-SMA, MHC and Smootheline) expression by UCs and SMCs respectively, over the period of 14 days culture. In conclusion, the ASC-based self-assembled scaffold seeded with UCs and SMCs has potential to be developed into a tissue-engineered human urethra for urethral reconstructive surgeries.

Keywords

Urethral Tissue Engineering ; Self-Assembled Scaffold ; Adipose-derived Stem Cell

Abstract **#1374**

COMBINATION OF PRINTING TECHNIQUES TOWARDS FABRICATION OF A BIOMIMETIC TYMPANIC MEMBRANE REPLACEMENT

Michael Gelinsky[P][1], Max von Witzleben[1], Thomas Stoppe[2], Matthias Bornitz[2], Marcus Neudert[2]

[1] *TU Dresden, Centre for Translational Bone, Joint and Soft Tissue Research*

[2] *TU Dresden, Department of Otorhinolaryngology, Head and Neck Surgery/Ear Research Center Dresden*

Corresponding author's email: michael.gelinsky@tu-dresden.de

Utilising MEW it is possible to create membranes with micrometre accuracy. However, the use of high voltage in the printing process imposes special challenges for the use of and the printing on support materials when compared to other 3D printing processes [1]. By combining fused deposition modelling (FDM), extrusion printing and MEW on one commercially available 3D printer, it was possible to reproduce the conical shape, the structural and acoustic properties of the human tympanic membrane. Extrusion printing was used to create a conical support structure over which the MEW fibres were deposited. FDM was used to print a ring around the MEW structure, which improved the handling of the scaffolds. The design of the support structure had significant influence on the placement of the individual MEW fibres and their diameter. Consequently, the mechanical properties of the manufactured scaffolds as well as the attachment and growth of keratinocytes on the scaffolds highly depended on the specific design of the support structure.

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Abstract **#1375**

CYTO-COMPATIBILITY AND BIOACTIVITY OF "SPRAYED" SILK FIBROIN ON HUMAN INTERVERTEBRAL DISC CELLS AND MESENCHYMAL STROMAL CELLS

Andreas Croft$_P$[1]

[1] *Tissue Engineering for Orthopaedics & Mechanobiology (TOM), Department for BioMedical Research (DBMR) of the Medical Faculty of the University of Bern, University of Bern, Bern, Switzerland.*

Corresponding author's email: andreas.croft@dbmr.unibe.ch

Intervertebral discs (IVD) are essential for six Degree-of-Freedom motion. However, a great drawback of an IVD is its minimal capacity for self-repairing. A promising approach to solve this problem could be the application of Bombyx mori silk. In this study, we aimed to test the cyto-compatibility and bioactivity of "sprayed" silk films in-vitro on primary patient-derived cells.

Human bone-marrow derived mesenchymal stromal cells (hBMSCs) were either seeded on a silk film or onto plastic as controls. Cells were then expanded for one week and subsequently cultured for 21 days with the addition of three growth factors (GFs); either TGFβ1, GDF6 or FGF2 to achieve a more disc-like phenotype. Then, the cell's metabolic activity, the DNA and glycosaminoglycans (GAGs) content and cell viability (CV) were analyzed.

HBMSCs' CV in medium containing TGFβ1 was higher on silk than on plastic, i.e., 92% vs. 66%.; with GDF6 it was 93% vs. 97%, and with FGF2 it was even reduced to 31% vs. 96%, respectively. In serum-free medium it was 96% and 95%, respectively. However, the cell's metabolic activity on silk was only between 36% and 65% of that on plastic after 21 days of culture, except for FGF2, where a 20% higher activity was observed on silk. Furthermore, cells cultured on silk produced half as many GAGs as the controls.

Current silk's cyto-compatibility revealed that fibers possibly should be functionalized with fibronectin or similar. Future work will focus on functionalization of the silk with specific growth factors and scaffold design.

Abstract **#1376**

SOX9 DETERMINES TRANSLATIONAL CAPACITY DURING EARLY CHONDROGENIC DIFFERENTIATION OF ATDC5 CELLS BY REGULATING EXPRESSION OF RIBOSOME BIOGENESIS FACTORS AND RIBOSOMAL PROTEINS

Marjolein M.J. Caron[1], Maxime Eveque[2], Berta Cillero-Pastor[3], Ron M. A. Heeren[2], Bas Housmans[1], Kasper Derks[4], Andy Cremers[1], Mandy J. Peffers[5], Lodewijk W. van Rhijn[1], Guus van den Akker[P1], Tim J.M. Welting[1]

[1] Laboratory for Experimental Orthopedics, Department of Orthopedic Surgery, Maastricht University Medical Center, Maastricht, the Netherlands

[2] Maastricht Multimodal Molecular Imaging institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University Medical Center, Maastricht, the Netherlands

[3] Maastricht Multimodal Molecular Imaging institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University Medical Center

[4] Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, the Netherlands

[5] Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom

Corresponding author's email: marjolein.caron@maastrichtuniversity.nl

In our previous research, we found that in addition to the well-known extracellular matrix-related expression of Sox9, chondrogenic differentiation of progenitor cells is driven by a defined bi-phasic expression of Sox9: an immediate early and a late (extracellular matrix associated) phase expression. In this study, we aimed to determine what biological processes are driven by Sox9 during the early phase of chondrogenic differentiation.

Sox9 expression in ATDC5 cells was knocked-down by siRNA transfection at the day before chondrogenic differentiation or at day 6. Samples were harvested at 2 hours, and 7 days in differentiation. The transcriptome (RNA-seq) and proteome (LC MS/MS) were compared using pathway and network analyses. Total protein translational capacity was evaluated with the SuNSET assay, active ribosomes with polysome profiling and ribosome modus with bicistronic reporter assays.

Early Sox9 knockdown severely inhibited chondrogenic differentiation weeks later. Sox9 expression during the immediate early phase of ATDC5 chondrogenic differentiation regulates the expression of ribosome biogenesis factors and ribosomal protein subunits. To validate functional consequences of siSox9 treatment for ribosome function, we evaluated total translational capacity, polysome profiles and ribosome modus. Translational capacity was decreased by siSox9 treatment and this correlated to lower amounts of active mono- and polysomes. Finally, cap- versus IRES-mediated translation was altered by Sox9 knockdown.

Here we identified an essential new function for Sox9 during early chondrogenic differentiation. A role for Sox9 in regulation of ribosome amount, activity and composition may be crucial in preparation for the demanding proliferative phase and subsequent extracellular matrix-production of chondroprogenitors.

Abstract **#1377**

3D BIOPRINTED COLLAGEN-GELATIN: BIOINKS FOR ADVANCED WOUND HEALING

Ali Smandri$_P$[1], Mh Busra Fauzi[1], Ng Min Hwei[1]

[1] *Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur 56000, Malaysia.*

Corresponding author's email: alialadil95@gmail.com

3D bioprinting has brought the biofabrication arena to an optimistic end by advancing the patterning approaches towards mimicking the extracellular matrix (ECM) of the native tissues. However, this approach is highly associated with the limitations of bioinks selection and availability. In wound healing, the major remaining challenge is the fast biodegradation of the existed bioinks post-implantation resulting in tissue regeneration failure, especially in large wounds. This study investigates the ability to print a hybrid of collagen-gelatin hydrogels (Col-Gel) for potential application in extensive wound healing. Col-Gel was first formulated printable by crosslinking with different concentrations of natural crosslinking agent (genipin). Further assessment was done by measuring the swelling ratio, viscosity, crosslinking degree, biodegradability, mechanical testing, and in vitro biocompatibility. The gross appearance results showed that the printed Col-Gel preserves high shape fidelity post-printing. The Col-Gel rheological properties were highly dependent on the crosslinking degree as increasing genipin concentrations leads to lower swelling ratio and viscosity. The biodegradation results confirm the ability of Col-Gel bioink to maintain its shape up to 14 days post-printing. Furthermore, the bioink showed over 90% cell viability with excellent cell adhesion and proliferation. Our results represent a new potential bioink for 3D bioprinting in wound healing.

Keywords

3D bioprinting; Skin regeneration; Collagen

Abstract **#1378**

EFFECTIVE IMMOBILIZATION OF BONE MORPHOGENETIC PROTEIN-2 (BMP-2) ON POLYCAPROLACTONE (PCL) NANOFIBERS USING NON-THERMAL PLASMA MODIFICATION FOR BONE TISSUE ENGINEERING

Mahtab Asadian[P][1,2], Gabriella Nilsson Hall[2,3], Iullia Onyshchenko[1], Rino Morent[1], Nathalie De Geyter[1], Lorenzo Moroni[4], Paul Wieringa[4], Ioannis Papantoniou[2,3,5], Liesbet Geris[2,3,6,7]

[1] *Research Unit Plasma Technology (RUPT), Department of Applied Physics, Ghent University*

[2] *Prometheus Division of Skeletal Tissue Engineering, Department of Materials Science, KU Leuven University*

[3] *Skeletal Biology and Engineering Research Center, KU Leuven, Belgium*

[4] *Department of Complex Tissue Regeneration, Maastricht University, The Netherlands*

[5] *Institute of Chemical Engineering Sciences, Foundation for Research and Technology – FORTH, Patras, Greece*

[6] *Biomechanics Research Unit, Université de Liège, Belgium*

[7] *Biomechanics Section, KU Leuven, Belgium*

Corresponding author's email: Mahtab.Asadian@UGent.be

Biomaterials for tissue engineering aim not only to possess adequate biodegradation rate, mechanical strength, and structural properties but also to effectively deliver specific growth factors to actively and timely guide cellular differentiation.

Polycaprolactone (PCL) meshes have been extensively used for bone tissue engineering due to their excellent mechanical properties and biocompatibility. However, PCL has high hydrophobic properties and lacks surface functional moieties, which often result in insufficient bone inductivity. Their bone-forming capacity can be improved by immobilization of bone morphogenic protein-2 (BMP-2), a strong osteoinductive factor, by various techniques. However, protein immobilization onto PCL is associated with challenges such as: (i) Impaired biological activity due to conformational changes of proteins during immobilization and (ii) influence of stability of BMP due to solvents used during the chemical conjugation processes. In this study, we aim to circumvent these problems by subjecting melt-electrowritten PCL meshes to low-pressure plasma treatment and subsequent exposure to an additional grafting procedure to immobilize BMP-2 onto the fiber surface. Surface characterization was performed using X-ray photoelectron spectroscopy for surface chemical analysis and scanning electron microscopy for morphological visualization. Surface analysis of the nanofibers confirmed the successful immobilization of protein. Moreover, luciferase reporter cells will be used to monitor the activation of BMP signaling. Taken together, we conclude that non-thermal plasma technology is a potent tool for protein immobilization and may be an effective modification method for improving and tailoring scaffold properties for bone defect regeneration.

Keywords

Bone tissue engineering; No-thermal plasma modification; Nanofiber

Abstract **#1379**

BODY-ON-A-CHIP MEDIA OPTIMIZATION WITH MACHINE LEARNING

Argus Sun$_P$[1,2], Nureddin Ashammakhi[3]

[1] *UCLA School of Engineering and Applied Sciences*

[2] *Eidolon Hydros*

[3] *UCLA Bioengineering*

Corresponding author's email: argus@eidolonhydros.com

Microphysiological systems (MPSs), also known as Organ-on-chip (OOC) are in vitro microfluidic models of organ function that attempt to mimic the in vivo microenvironment of the component tissues of an organ. However, when applied to toxicity testing of pharmaceuticals, multi-organ interactions are sometimes necessary. Cocultures or Body-on-chip (BOC) can link up to 10 MPSs microfluidically[1]. This brings two important challenges, the pharmacokinetic tissue:tissue and tissue:media ratio, as well as the need for a universal media formulation that will provide optimum cell growth in all linked microenvironments [2] Machine Learning and Artificial Intelligence methods have been proposed to address these challenges[3]. We address the latter by constructing a stoichiometric matrix of 10 commercially available cell culture medias. Using machine learning algorithms we show that optimal universal media formulations can be found for several configurations of body-on-chip systems.

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Abstract **#1380**

3D BIOPRINTED VASCULARIZED-BONE-CHIPS: MULTIMATERIAL INTERPHASES IN BONE IN VITRO MODELS

Alvaro Sanchez-Rubio$_P$[1], Vineetha Jayawarna[1], Matthew J. Dalby[1], Manuel Salmeron-Sanchez[1]
[1] *Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, United Kingdom*
Corresponding author's email: a.sanchez-rubio.1@research.gla.ac.uk

Bone-associated diseases account for more than 145 billion euros per year in the US and Europe alone, so being able to understand underlying mechanisms and having reliable models is key in the future of not only medicine and pharma but also economy and society. In vitro models are a widely used tool in order to explore diseases, biological processes or for drug-screening purposes. However, traditional 2D models lack most of the interactions present in in vivo microenvironments whereas existing 3D models lack the geometrical, heterogeneity and spatial distribution needed to better mimic bone architectures. 3D Bioprinting, which uses Computer Aided Design (CAD) to deposit chosen materials and biological components in predefined architectures emerges as a key tool for achieving higher complexity models that could integrate both 3D culture features, along with specific material properties or cell types (co-culture) in different compartmentalized areas. In this work we propose a customizable 3D Bioprinting platform that can be used to reproducibly manufacture high-throughput multimaterial interphases in in vitro models granting control over special physical, chemical and biological properties, and its application for vascularized bone studies. These interphase-based vascularized-bone-chips are composed of an inner circular region, with stiffnesses below 10 kPa which holds HUVEC cells stimulated by VEGF, surrounded by an outer ring, with stiffnesses over 30 kPa that contains BMP-2 to stimulate osteogenesis of hMSCs cells. The model emulates the endothelial and osseous components of bone, which we will use to investigate appropriate phenotyping and effect of interphases in paracrine signaling between compartments.

Keywords
in vitro model; vascularized bone; interphase

Abstract **#1382**

A HUMAN-BASED 3D PLATFORM TO SUPPORT OSTEOSARCOMA SPHEROID INVASION IN A TRI-CULTURE ENVIRONMENT FOR DISEASE MODELLING AND DRUG SCREENING APPLICATIONS

Cátia F. Monteiro[P][1], Catarina A. Custódio[1], João F. Mano[1]
[1] *Department of Chemistry, CICECO, University of Aveiro Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*
Corresponding author's email: cfrmonteiro@ua.pt

The high failure rate of osteosarcoma (OS) drug discovery is steadily increasing owning to the limited predictability of the current preclinical models. Advances in functional and biomimetic materials from natural or synthetic origin have been contributed to the faithful recapitulation of a broad range of diseases' pathophysiology.1,2 In this scope, the potential of methacryloyl platelet lysates (PLMA)-based hydrogels for cell culture purposes3,4 are herein explored to establish an OS tri-culture model for tumor invasiveness studies and drug screening.
The architecture and synergistic interaction of an invading OS tumor was recapitulated encapsulating OS spheroids in PLMA hydrogels, alone or co-cultured with human osteoblasts and human bone-marrow mesenchymal stem cells (hBM-MSC). PLMA hydrogels supported tumor growth and the formation of tumor invasive branches from the spheroid in both settings. However, the presence of surrounding stromal cells potentiated tumor invasiveness ability into the ECM-mimicking matrix. The deposition of bone-related proteins and minerals were also observed. The exposure of the established models to doxorubicin revealed a higher IC50-value of PLMA-based models, comparing with scaffold-free spheroids. The proposed 3D OS models highlighted the potential of PLMA hydrogels to support an invasive tumor behavior and recapitulate tumor-stromal cell crosstalk, exhibiting a physiologically predictive microenvironment for OS drug discovery.

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Keywords
Osteosarcoma; Human-derived hydrogel; Invasion modeling

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Abstract **#1383**

3D-PRINTING OF GELATIN BASED SCAFFOLDS FOR TISSUE REGENERATION: PROCESSING AND CHARACTERIZATION

Petar Uskokovic[P][1], Marija Jovanovic[1], Milos Petrovic[1], Dusica Stojanovic[1], Vesna Radojevic[1]
[1] *University of Belgrade - Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Serbia*
Corresponding author's email: marija.jovanovic@tmf.bg.ac.rs

The scaffolds based on natural polymers can be used for various treatments of bone injuries. This scaffold loaded with biological factors or active substances can lead to bone regeneration and suppress the patient's pain or the immune response. Gelatin is a promising polymer for this application because of its biocompatibility, biodegradability and availability, but it has poor mechanical properties. To overcome this problem we blended gelatin with polyvinylpyrrolidone (PVP) because of its amorphous structure and favorable adhesive behavior. The synthesized scaffold was cross-linked with glutaraldehyde and lyophilized. We also added ibuprofen to reduce pain and tissue inflammation. The aim of this work was to optimize the composition of scaffold fabricated by semi-solid 3D printing, as well as to examine the impact of PVP and ibuprofen on the mechanical and thermal properties of fabricated scaffolds. Chemical interactions during processing were investigated by FTIR spectroscopy. Thermal analysis of scaffold was done using DSC method. Scaffold morphology was followed by SEM analysis. In vitro drug release study under physiological conditions was performed to investigate the influence of gelatin/PVP ratio and scaffold morphology on drug release profiles. Mechanical properties such as hardness, toughness, tensile strength and bone adhesion were investigated by Texture Analyzer.

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Abstract **#1385**

FROM CLASSICAL BIOMATERIALS TO MODERN CONCEPTS FOR BONE AND OSTEOCHONDRAL TISSUE ENGINEERING – DEVELOPMENT OF MODULAR MATERIAL SYSTEMS FOR 3D (BIO)PRINTING

Anja LodeP1

[1] *Technische Universitaet Dresden, Centre for Translational Bone, Joint and Soft Tissue Research*

Corresponding author's email: anja.lode@tu-dresden.de

The advent of additive manufacturing technologies in tissue engineering has revolutionized this research field by strongly extending the options for the development of complex tissue substitutes and models. In the last years, enormous progress was made to adapt biomaterials for 3D printing technologies and to refine the techniques to allow bioprinting – the integration of biological components, i.e. cells and/or growth factors, in the fabrication process. Extrusion-based 3D (bio)printing has great potential for skeletal applications as volumetric constructs with clinically relevant dimensions can be produced in an appropriate time window and various biomaterials and/or multiple cell types can be combined within one construct by using multi-channel tools.

This presentation describes the development of modular material systems suitable for extrusion 3D (bio)printing: Based on classical bone replacement materials, self-setting calcium phosphate cements (CPC) and mesoporous bioactive glasses (MBG), printing materials were generated with tailorable degradation properties and various drug delivery functions. Bioinks with excellent printing fidelity, consisting of alginate and methylcellulose, were functionalized with microparticles (e.g. MBG or magnetite), nanoclays (e.g. Laponite) or natural matrix components to obtain growth factor delivery and cell supportive features or to allow magnetically actuated mechanical stimulation of the embedded cells. Methods for combined processing of bioinks and CPC-based printing materials, by using tandem or core/shell printing, were established and successfully applied towards the fabrication of individualized, complex bone and osteochondral implants.

Abstract **#1387**

AN ENGINEERED HUMAN OSTEOSARCOMA-ON-A-CHIP FOR TUMOR INVASION MODELLING IN A DYNAMIC SYSTEM

Cátia F. Monteiro$_P$[1], Catarina A. Custódio[1], João F. Mano[1]

[1] *Department of Chemistry, CICECO, University of Aveiro Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*

Corresponding author's email: cfrmonteiro@ua.pt

In vitro tumor invasion modelling combining 3D biomimetic materials and multicellular spheroids has sought to achieve the complexity of tumor microenvironment, including for osteosarcoma (OS).1-3 However, the lack of mechanical stimuli, tumor-stromal cell interaction and tumor angiogenesis are critical limitations. Recent advances in engineered organ-on-a-chip platforms have revealed the potential of these microsystems to recapitulate pathological features.4,5 In an attempt to provide a dynamic platform for OS tumor invasion modelling, the aim of this study is the development of a human osteosarcoma-on-a-chip to establish the vasculature-bone tumor barrier.

A microfluidic chip was designed to comprise a channel to introduce an ECM-mimicking matrix and perfusable channels to recreate vascular vessels through endothelial cell culture. Exploring a fully human-derived approach, MG-63 tumor spheroid was embedded in human methacryloyl platelet lysates (PLMA)-based hydrogel. The synergistic tumor-stromal cell and cell-extracellular matrix interaction of an invading tumor was studied in mono- and co-culture configurations. As result, the tumor growth was supported and an increased spheroid invasion into the co-culture model was verified. The development of microcapillary-like protrusions into the hydrogel was also evaluated, opening up the opportunity to recreate the tumor intravasation process. The presented human 3D dynamic model provides the first osteosarcoma-on-a-chip to study tumor progression events, offering an innovative platform for anticancer therapies screening.

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Keywords

Osteosarcoma; Human-derived hydrogel; Invasion modeling

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Abstract **#1388**

EFFECT OF INNERVATION ON PANCREAS INFLAMMATION

Adrián Seijas Gamardo[P][1], Lorenzo Moroni[1], Paul Wieringa[1]

[1] MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration Department, Maastricht University, The Netherlands

Corresponding author's email: a.seijasgamardo@maastrichtuniversity.nl

Patients that undergo an islet transplantation suffer from 50% loss of transplanted islet mass postintervention, during engraftment period. Among other causes inflammation plays a detrimental effect on them. Here, we present endogenous innervation as a novel regenerative medicine approach to improve tissue implantation outcomes. During islet transplantation in diabetic patients, several proinflammatory cytokines are expressed, including interleukin 1ß (IL-1β), tumor necrosis factor-a (TNF-α) and interferon-g (IFN-γ). This inflammation decreases the viability and functionality of islets, negatively affecting transplantation outcomes. Transplanted pancreatic islets can become innervated depending on the transplantation site. These neurons influence cell behavior by secreting neuropeptides, such as substance P (SP), vasoactive intestinal peptide (VIP), or calcitonin gene-related peptide (CGRP). Little is known about the effect of these neuropeptides on pancreas inflammation. However, understanding which cytokine combinations lead to cell death pathways, and the neuropeptides that modulate this response, will help understanding their mechanism of action. Thus, improving the outcome of islet transplantation. In this study, we cultured INS1E cells and assessed cell viability after exposing them to pro-inflammatory cytokines in different combinations with neuropeptides. Pseudoislets were also cocultured with neurons derived from iPSCs in order to show the innervation that can occur after transplantation. We confirmed the detrimental effect of cytokines when applied to cell cultures and observed mitigation of these effects in the presence of neuropeptides. These results suggest that a correct innervation is key for maintaining homeostasis in the pancreas and give insights into mechanisms involved in cell survival, informing future strategies for tissue implantation.

Keywords

Pancreas; Innervation; Inflammation

Abstract **#1389**

MULTIFACETED POLYACTIVE® GUIDANCE CONDUITS WITH DISTINCTIVE DOUBLE-LAYERED ARCHITECTURE AND PLASMA-INDUCED INNER CHEMISTRY GRADIENT FOR THE REPAIR OF CRITICAL-SIZED NERVE DEFECTS

Rouba Ghobeira[P][1], Paul Wieringa[2], Stijn Van Vrekhem[1], Sheida Aliakbarshirazi[1], Iuliia Onyshchenko[1], Nathalie De Geyter[1], Lorenzo Moroni[2], Rino Morent[1]

[1] *Research Unit Plasma Technology (RUPT), Department of Applied Physics, Faculty of Engineering and Architecture, Ghent University, Ghent, Belgium*

[2] *Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands*

Corresponding author's email: Rouba.Ghobeira@Ugent.be

Despite all tissue engineering advances, the lately designed nerve guidance conduits (NGCs) are still failing in repairing critical-sized nerve defects. Our goal was therefore to tackle large nerve gaps (2 cm) by designing an NGG possessing refined physicochemical properties enhancing Schwann cell (SC) performances and thus triggering a potent nerve regenerative capacity. To do so, a combinatorial strategy adopting novel plasma-induced surface chemistry and architectural heterogeneity was considered. A mechanically suitable copolymer (Polyactive®) was selected and electrospun to produce nanofibrous NGCs mimicking the neural extracellular matrix. An innovative double-layered architecture consisting of an inner wall comprised of bundles of aligned fibers with intercalated random fibers and an outer wall fully composed of random fibers was conceived to synergistically provide cell guidance cues and a sufficient nutrient inflow. NGCs were then subjected to argon plasma treatments making use of a dielectric barrier discharge (DBD) and a plasma jet (PJ). The DBD treatment homogeneously increased the inner O-content from 17% to 28%. The PJ treatment created a chemistry gradient with an O-content gradually increasing from 21% to 30% along the inner NGC wall. In vitro studies revealed enhanced SC adhesion and elongation on plasma-treated NGCs. Interestingly, a cell gradient was observed on PJ-treated NGCs thus underlining the favorable chemistry gradient in promoting cell chemotaxis towards high O-contents. A gradual change from circular to highly elongated SC morphologies mimicking the natural bands of Büngner was visualized along the gradient. Overall, plasma-treated NGCs are promising candidates paving the way towards critical nerve gap repair.

Keywords

Nerve guidance conduits; Electrospinning; Plasma surface treatment

Abstract **#1390**

A CONTINUOUS MICROFIBER WIRE MANDREL-LESS BIOFABRICATION FOR SOFT TISSUE ENGINEERING APPLICATIONS

Arianna Adamo[P][1,2], Joseph B. Bartolacci[3], Marco Traina[1], William R. Wagner[3], Antonio D'Amore[3,4]

[1] *University of Palermo, Palermo, Italy*

[2] *3Departments of Bioengineering and & Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, USA*

[3] *Departments of Bioengineering and & Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, USA*

[4] *RiMED Foundation, Palermo, Italy*

Corresponding author's email: arianna_adamo@hotmail.it

Soft tissue injuries are common in daily clinical and surgical practice. Outcomes of degradable and non degradable suture materials are often affected by mechanical mismatch, excessive fibrosis and inflammation. This study introduces a mandrel-less electrodeposition method able to fabricate continuous microfiber wires, with controlled micro-architecture and tunable mechanics. Poly(ester urethane) urea (PEUU) microfiber wire morphology and mechanical properties have been characterized by scanning electron microscopy uniaxial tensile test respectively Furthermore, the in vitro response of mouse bone marrow-derived macrophages to PEUU degradation products, PEUU electrospun and casted scaffold, PEUU electrospun wires was evaluated by immunoblotting and immunolabeling. Moreover, the host response to electrospun wires in vivo was tested: twenty rats, randomized in 5 groups, received a 2cm infra-scapular incision and the skin was closed using PEUU microfiber and cast wires and the most common suture materials used (polyglycolic acid, polydioxanone and polypropylene). After one month, mechanical and histological evaluation of explants and suture wires was performed. In vitro results have shown an anti-inflammatory macrophage response associated to PEUU microfiber scaffold and wires. In vivo, PEUU electrospun wires group showed better mechanical performance compared to the other groups, a favorable collagen remodeling comparable to the healthy group and a mild host response reaction. These results suggest that microfiber wires reduce macrophage pro-inflammatory response and improve collagen deposition, which make it an ideal candidate for soft tissue suture applications.

Abstract **#1391**

ADENOSINE RELEASE FROM MICROPARTICLES FOR BONE REGENERATION

Hadi Hajiali$_P$[1], Manuel Salmerón-Sánchez[2], Matthew J. Dalby[2], Felicity R. A.J. Rose[1]

[1] *Division of Regenerative Medicine and Cellular Therapies, Nottingham Biodiscovery Institute, School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK.*

[2] *Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, G12 8LT, UK.*

Corresponding author's email: hadi.hajiali@nottingham.ac.uk

There have been many studies conducted into the delivery of factors, such as small molecules, to support and augment new bone formation. It is essential to control the spatio-temporal release kinetics of such factors in order to enhance their efficiency and reduce the side effects of their high dose [1]. This study aimed to investigate the effect of adenosine release from microparticles designed for local release of adenosine for bone tissue engineering applications. Polymer microparticles (MPs) were formed using a solid-in-oil-in water(s/o/w) emulsion based on a previously published protocol [1]. Primary human mesenchymal stem cells (MSCs; Batch No: 18TL262066 from Lonza) were seeded into MPs sintered to form disks and cultured in osteogenic induction media. Alizarin Red staining were conducted after 3 weeks to evaluate the mineralisation and hence differentiation of MSCs to the osteoblast lineage. Alizarin Red staining and quantification analyses illustrated a significant increase ($p < 0.0001$) in mineralisation when MSCs were cultured in the MPs releasing adenosine after 3 weeks of induction. Microscopy imaging also demonstrated improvement of calcification around the cells in the PLAGA/Ad samples. These data clearly illustrate the beneficial effect of release of adenosine from MPs in the osteogenic differentiation of MSCs on these microparticles. Further in vivo studies will investigate osteoinductivity of PLGA/Ad MPs when implanted in critical-sized bone defects.

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Abstract **#1392**

ENGINEERING ORTHOPAEDIC CELL THERAPIES: TRANSLATION TO THE CLINIC

Alicia El Haj[P][1,2]

[1] *Healthcare Technology Institute*

[2] *University of Birmingham*

Corresponding author's email: a.elhaj@bham.ac.uk

Regenerative medicine principles are based on the power of progenitor cells to regenerate and grow tissues in vitro and in vivo. Engineering provides the platforms and tools which enable the control and manufacture of cells used for clinical therapy and regenerative models. Biological and physical factors play a key role in enabling the cell niche which supports the capability for rebuilding tissue complexity. Using our knowledge of controlling in vitro tissue growth, we can begin to engineer new ways to implement regenerative approaches in vivo for treating patients. One aspect of this is how to control injectable cell therapies, which can then be used routinely within a hospital or primary care environment.

Here, we describe a novel bio-magnetic technology, MICA, where magnetic nanoparticles (MNPs) are used to remotely deliver mechanical stimuli to the cell-surface mechano-receptor, TREK-1, resulting in activation and downstream signalling via an external magnetic array which can be used for multimodal targeting and imaging. In these studies, we have translated MICA to a pre-clinical ovine model of bone injury to evaluate functional bone repair. We present evidence to support early accelerated repair and preliminary enhanced bone growth in MICA-activated defects within individuals compared to internal controls. The variability in donor responses to MICA-activation was evaluated in vitro revealing a clear relationship between responders to MICA in vitro and in vivo. These unique experiments offer exciting clinical applications. Translation and commercialisation of this 'novel' technology presents challenges in regulatory and clinical adoption which will be described.

Abstract **#1393**

OSTEOINDUCTIVE PERFORMANCE OF DECELLULARIZED TISSUE ENGINEERED CARTILAGE

Alejandro Garcia Garcia[P][1,2], Sujeethkumar Prithiviraj[3,4], Sebastien Pigeot[5], Deepak B. Raina[6,7], Ivan Martin[5], Paul E. Bourgine[3,2]

[1] *Laboratory for Cell, Tissue and Organ engineering, Lund stem cell center, Lund University, Sweden*

[2] *Wallenberg Centre for Molecular Medicine at Lund University, Sweden*

[3] *Laboratory for Cell, Tissue and Organ engineering, Lund stem cell center, Lund University, Sweden.*

[4] *Wallenberg Centre for Molecular Medicine at Lund University, Sweden.*

[5] *Department of Biomedicine, University Hospital Basel, University of Basel, 4031 Basel, Switzerland.*

[6] *Department of Orthopedics, Lund University, Sweden*

[7] *Clinical and Experimental bone healing.*

Corresponding author's email: alejandro.garcia@med.lu.se

Introduction: Engineered and decellularized extracellular matrices (dECM) are receiving increasing interest in bone tissue engineering. Current limits mainly arise from the important variability of performance which can result from the use of primary cells but also from the decellularization method. In this study we propose to overcome these barriers using a customized human mesenchymal stromal cell line (hMSCs) capable of reproducible cartilage formation. We here investigate the possibility to efficiently decellularized our cartilage graft while maintaining its osteo-inductive capacity.

Method: Our graft consists of in vitro engineered cartilage tissue produced by the hMSCs. Subsequently, the tissue is decellularized by a combination of hypertonic/hypotonic, detergent (SDS) and DNase washes. To assess the capacity to instruct bone formation by endochondral ossification, the decellularized constructs are implanted subcutaneously in the back of ID mice for 12 weeks. Readouts consists in histochemistry, biochemical analysis and micro computed tomography (µCT).

Results: We demonstrated the reproducible engineering of decellularized cartilage by exploitation of a mesenchymal line. Decellularization resulted in a drastic reduction of DNA (96%) with a minimal impact on tissue structure and composition (collagen, GAGS and embedded growth factors). After 12 weeks in vivo, extensive tissue remodelling was found on decellularized material as confirmed by µCT.

Conclusion and discussion: Our study illustrates the capacity of exploiting customized human lines to produce osteoinductive dECM. The strategy offers both reproducibility of performance and unlimited tissue availability, opening new avenues for the manufacturing of dECM for bone repair.

Keywords Bone, Tissue Engineering, Decellularization.

Abstract **#1394**

CHEMICALLY DECORATED DECELLULARIZED CORNEA AS FULL-THICKNESS STROMAL SUBSTITUTE

Juhi Chakraborty[P][1], Subhadeep Roy[1], Sourabh Ghosh[1]

[1] *Indian Institute of Technology Delhi*

Corresponding author's email: chakraborty.juhi21@gmail.com

During the ongoing Covid-19 pandemic, collection and donation of human cadaveric corneas are cumbersome. Decellularized corneas (DC) have gained intense popularity as a possible scaffold for corneal remodeling and as an alternative tissue source for corneal replacement. However, DC elicits immune response inspite of elimination of the cellular contents/antigens due to distortion of the collagen fibrils that exposes certain antigenic sites, which often lead to graft rejection. Therefore, here, we tested the hypothesis that cross-linking DC with chondroitin sulfate (CS) may help in restoring distorted conformational changes of the fibrous matrix and would reduce graft rejection. An in vitro immune response study confirmed that the cross-linked DC elicited the least immune response than DC. We implanted three sets of corneal scaffolds obtained from goat, i.e., native, decellularized, and DC conjugated with CS into rabbit stroma. Histology analysis, three months post-implantation confirmed seamless graft integration, cell migration, and no sign of inflammation in the crosslinked cornea. However, so far we have checked the immunogenic potential of decellularized and crosslinked cornea among cross-species(goat to rabbit). Now, before moving to a human clinical trial (patients with infectious keratitis), we are validating the decellularization of the human stromal layer using discarded human corneas not suitable for implantation, for the regeneration of the corneal endothelial layer. The decellularized, chemically decorated cornea will be tectonically strong, offer less immunogenicity, can be sterilized, and will have a longer shelf life. Through this novel study, we can meet the demand for alternative bioengineered human cornea for keratitis patients.

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Abstract **#1395**

BIOMIMETIC ENGINEERED CHORDAE TENDINEAE GENERATED WITH MANDREL-LESS FABRICATION

Arianna Adamo[P][1,2], Giovanni Spiaggia[3], William R. Wagner[2], Antonio D'Amore[2,4]

[1] *University of Palermo, Palermo, Italy*

[2] *Departments of Bioengineering and & Surgery, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, USA*

[3] *Adolphe Merkle Institute, University of Fribourg, Fribourg, CH*

[4] *RiMED Foundation, Palermo, Italy*

Corresponding author's email: arianna_adamo@hotmail.it

Current methods for chordae tendineae (CT) replacement do not recapitulate the micro-architecture nor the mechanics of the native CT and do not promote healthy tissue regeneration. This study introduces a mandrel-less electrodeposition methodology to create a biomimetic engineered CT (BECT) using a bio-absorbable polymer. Human and porcine CT were harvested and tested to evaluate mechanical and histological native tissue structure. Poly(carbonate-urethane) urea was used to fabricate BECT via mandrel-less electrodeposition. Moreover, BECT were micro-integrated with NIH-3T3 rat fibroblasts. Micro-integrated BECT were divided in 3 groups: static culture in plate, tension culture in plate and dynamic culture using a stretch bioreactor. All groups were conditioned for 1 week. Finally, scanning electron microscopy (SEM), uniaxial tensile testing and histological evaluation were performed to compare native tissue and BECT micro-architecture and mechanical properties. BECT mimicked native CT shape, diameter, and length. SEM analysis showed highly aligned fiber microstructure recapitulating the arrangement observed in native CT. BECT mechanical characterization showed a lower elastic modulus than native tissue that increased with dynamic conditioning for cell microintegrated BECT. The histology of cell-seeded BECT demonstrated cell adhesion and infiltration after one week. This mandrel-less electrodeposition method allows the bio-fabrication of BECT that demonstrate the ability to recapitulate native CT structure with use of a scaffold. Micro-integration preliminary data provided evidence of cell proliferation and viability, demonstrating an early proof-of-concept for potential host cell recruitment.

Abstract **#1396**

THE COMPARATIVE EFFECTS OF MESENCHYMAL STEM CELL TRANSPLANTATION THERAPY FOR SPINAL CORD INJURY IN HUMANS AND ANIMAL MODELS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Louis Johnson[P1], Eustace Johnson[1]

[1] *Chester Medical School, University of Chester, UK*

Corresponding author's email: eustace.johnson@chester.ac.uk

Animal models have been used in preclinical research to examine potential new treatments for spinal cord injury (SCI), including mesenchymal stem cell (MSC) transplantation. MSC transplants also have been investigated in early clinical human studies and trials. Whether and how well the animal studies inform and represent the human outcomes is unclear. In this study, we have completed a systematic review and meta-analysis to examine the effects of MSC transplants on functional outcomes in humans and animal models (mostly rats) of SCI. Following defined searches in PubMed, clinicaltrials.gov and the Cochrane library, identified papers were screened and available data extracted and analysed. The findings are promising. MSC transplantation was associated with significantly improved American Spinal Injury Association (ASIA) motor and sensory scores in humans and significantly increased locomotor function scores in animals. However, there were major discrepancies between the human and animal studies, including timing of MSC transplant, cell source, scale, modes of MSC delivery, and reports of adverse events. Additionally, difficulties in the comparing functional outcome measures across species limit the predictive nature of the animal research. Previously, we reported on the neurotrophic effects of canine MSCs [1], demonstrated that these activities are influenced by the CNS wound microenvironment [2], and, recently, quantitatively compared the neurotrophic effects of canine and human MSCs [3]. The findings of the systematic review and meta-analysis will be considered in the context of these recent papers, with recommendations for research that better enables the translation of animal models of SCI to human MSC-based therapies.

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Abstract **#1397**

APPLICATION OF THE 'UPSIDE DOWN' CHIP TO MODEL CARDIOMYOPATHY ASSOCIATED WITH DUCHENNE'S MUSCULAR DYSTROPHY

Aoife Gowran[P][1], Stefano Piazza[1,2], Roberta Visone[2], Elisa Castiglioni[1], Giulio Pompilio[1,3], Alberto Redaelli[2], Marco Rasponi[2]

[1] *Unit of Vascular Biology and Regenerative Medicine, Centro Cardiologico Monzino-IRCCS, Milan, Italy*

[2] *Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy*

[3] *Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Italy*

Corresponding author's email: agowran@ccfm.it

This study combined Duchenne's muscular dystrophy (DMD) patients' induced pluripotent stem cells (iPSCs) from Centro Cardiologico Monzino's Cardiovascular Biorepository with a re-design of the beating-heart-on-a-chip developed by Marsano et al [1]. DMD is a genetic disease caused by dystrophin gene mutations that frequently cause juvenile-onset cardiomyopathy. Specifically, cardiomyocytes from DMD patients' iPSCs (iPS-CMs) were cultured in the new 'upside down' (UD) chip which consists of two layers: an upper actuation layer that transfers mechanical stretch to cells within a 3D fibrin hydrogel housed in the lower layer bonded to a glass coverslip. Healthy and DMD iPS-CMs harvested from the 2D cultures were transferred to the UD chip and used to optimize: i) day of harvesting from 2D, ii) cell aggregation level (single cells-SC or small aggregates-SA), and iii) fibrin gel concentration (7.5, 10, 20 mg/ml). Cultures were maintained under dynamic conditions (0.5 bar, 1 Hz, 50% duty cycle, 4h post-seeding) and cell viability was assessed after 0, 7 and 14 days. The optimal culture parameters for healthy and DMD iPS-CMs were observed for iPS-CMs harvested on day 17, triturated as SA and seeded in 7.5 mg/ml fibrin. Healthy iPS-CM maturity was demonstrated in the UD chip by high-resolution cardiac troponin T imaging and field potential electrical recordings. The platform is versatile being also suitable for co-cultures and ELISA of cell-conditioned media. Thus it could be exploited for disease modelling to further investigate DMD disease readouts, e.g. calcium dynamics, and gauge the impact of potentially therapeutic drugs.

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Abstract **#1398**

PROFILING IMMUNE CELL-MATERIAL INTERACTIONS IN INJURY MODELS THROUGH QUANTITATIVE CELL-LEVEL ANALYSES

Ying Luo[P][1], Fang Wang[1], Ni Su[1]

[1] *Department of Biomedical Engineering, Peking University*

Corresponding author's email: yluo.pku@gmail.com

Biomaterials have found important applications as carriers of biological entities for developing next-generation regenerative therapies. However, biomaterial mechanisms and associated functions remain to be elucidated within the context of the immune responses induced in the body. Here we investigate two biomaterial-based systems respectively carrying small RNA molecules and exosome vesicles and report systematic cell-level analyses to understand how cargo biologics were interacting with and taken up by different types of cells in the host tissue. First, polyamindoamine dendrimeric nano materials complexed with small RNA molecules were studied in injured heart tissue in zebrafish. The whole-heart analyses indicated that non-macrophage leukocytes at day 1 and 7 ingested close to 50% RNAs, followed by macrophages, endothelial cells and cardiomyocytes in the injured area. In another system, fluorophore-labeled exosomes derived from mensenchymal stromal cells (MSCs) were immobilized to fibrous polycaprolactone scaffolds and were studied in skin wound-healing models in mice. The analyses on the wounded tissues treated by scaffolds revealed that the exosome vesicles on day 7 were mainly taken up by macrophages at 70% level while the macrophage population accounted for 25% of the total cells. In responding to MSC exosomes, the macrophages in wounds were polarized to the M2 anti-inflammatory subtype, which could further induce beneficial adaptive immune responses. Our study suggest that immune cells were major responders to biomaterial-based systems and mediated heterogenous reactions to affect the delivery pattern and tissue responses. The immune cell-mediated reactions have important implications for guiding the function design of biomaterials.

Abstract **#1399**

IN VITRO BLASTOCYST IMPLANTATION MODEL BY THE USE OF SYNTHETIC POLYMER FOAM SCAFFOLDS

Deniz Basoz[P][1], Yagmur Ergun[2], Ahmet Kocabay[3], Ali Cihan Taskin[3], Deniz Yucel[1,4], Sercin Karahuseyinoglu[2,5]

[1] Department of Medical Biotechnology, Graduate School of Health Sciences, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey;

[2] Department of Reproductive Biology, Graduate School of Health Sciences, Koç University, Istanbul, Turkey

[3] Animal Research Lab, Koç University Research Center For Translational Medicine (KUTTAM)

[4] Department of Histology and Embryology, School of Medicine, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey

[5] Department of Histology and Embryology, School of Medicine, Koç University, Istanbul, Turkey

Corresponding author's email: denizbasoz@gmail.com

Implantation is a complex and interactive event between endometrium and hatched blastocyst. The success of implantation depends on the synchronization of endometrium receptivity as well as the capacity of the blastocyst to implant. Use of embryo transfer media enriched with extracellular matrix components or pre-activation of endometrium might not be sufficiently effective for embryo transfer. The aim of this study was to mimic 3D blastocyst implantation environment with endometrial cells seeded onto synthetic polymer foams. The scaffolds were prepared with a blend of poly(D,L-lactide-co-glycolide) and poly(L-lactide-co-D,L-lactide) at different concentrations and lyophilized. The morpholgy and pore size of the foams were investigated with scanning electron microscope. It was observed that the polymer concentration and the freezing temperature before lyophilization affected the pore size of foams. It was decided to use foams with about 60µm pore size in in vitro studies. The foams were coated with collagen, and then seeded with endometrial (epithelial and stroma) cells. Mouse blastocysts were seeded on this construct, and incubated for 48 and 96 hours. The expression of certain implantation markers was investigated by qRT-PCR. It was observed that the expression of genes related with adhesion and invasion like L-selectin, laminin, E-cadherin, collagen IV, integrin, MMP9 were significantly altered within 48-96 hours. The results showed that synthetic polymer foams with endometrial co-culture would be a potential 3D in vitro blastocyst implantation model by supporting adhesion and invasion phases of implantation. Acknowledgements: This study was supported by The Scientific Technological Research Council of Turkey (TUBITAK) SBAG 116S527.

Abstract **#1400**

FORMATION OF LARGE MUSCLE GRAFTS AND SUBSTANTIAL IMPROVEMENT OF HEART FUNCTION AFTER INJECTION OF HIPSC-CARDIOMYOCYTE AGGREGATES IN A NON-HUMAN PRIMATE MODEL OF MYOCARDIAL INFARCTION

Ina Gruh[1,2,3], Serghei Cebotari[4,2], Andreas Martens[4,2], Wiebke Triebert[1,2,3], Alexandra Haase[1,2,3], Annette Schrod[5], Amir Moussavi[5], Vanessa Neuhaus[6], Christian Veltmann[7,2], Danny Jonigk[8,2], Susann Boretius[5], Armin Braun[6], Axel Haverich[4,2,3], Robert Zweigerdt[1,2,3], Ulrich Martin[1,2,3]

[1] *Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO)*

[2] *Hannover Medical School, Hannover, Germany*

[3] *REBIRTH Research Center for Translational Regenerative Medicine*

[4] *Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG)*

[5] *Leibniz Institute for Primate Research, Goettingen, Germany*

[6] *Fraunhofer ITEM, Hannover, Germany*

[7] *Department of Cardiology and Angiology*

[8] *Institute of Pathology*

Corresponding author's email: gruh.ina@mh-hannover.de

Background and aim – Therapeutic application of hiPSC-derived cardiomyocytes (CMs) is considered a promising approach for heart repair. We hypothesized that injection of human iPSC-CM aggregates (hiCMAs) leads to improved cell retention and superior engraftment compared to single cell injection. It was our aim to investigate the regenerative potential of hiCMAs after sub-acute myocardial infarction in a pre-clinical NHP model.

Methods – Myocardial infarction (MI) was induced by coronary artery ligation in cynomolgus monkeys (Macaca fascicularis; n=11). Cardiac function was assessed via telemetric ECG recording, echocardiography and MRI. hiCMAs expressing a fluorescent reporter gene were generated by targeted differentiation in large-scale suspension cultures. 5 - 7 x 10 7 hiPSC-CMs were injected directly into the myocardium 2 weeks after MI. 2 weeks or 12 weeks later, animals were sacrificed and graft survival and cell distribution was assessed histologically.

Results and Conclusion – Both 2 weeks and 12 weeks after cell transplantation, large hiPSC-CM grafts (>1 mm2) were identified in myocardial tissue sections based on their reporter gene expression and after staining with a species-specific antibody targeting human cardiac troponin I. Human iPS-CMs showed alignment and expressed cardiac markers, visible cross striations reflected sarcomeric structures. After hiCMA transplantation, MRI data showed a remarkable functional improvement of LVEF after 12 weeks (n=3), in contrast to the non-treated control. In conclusion, we demonstrated formation of large hiCMA-derived heart muscle grafts 2 and 12 weeks after transplantation in our pre-clinical NHP model associated with a substantial functional improvement.

Abstract **#1401**

HOMOGENISATION OF BIOINSPIRED VOXEL-BASED COMPOSITES VIA MACHINE LEARNING ESTIMATION

Mauricio Cruz Saldivar$_P$[1], Mohammad J Mirzaali[1], Eugeni L Doubrovski[2], Amir A Zadpoor[1]

[1] Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology (TU Delft), The Netherlands

[2] Faculty of Industrial Design Engineering (IDE), Delft University of Technology (TU Delft), The Netherlands

Corresponding author's email: m.cruzsaldivar@tudelft.nl

Composite design via Voxel-based methods allows manufacturing a wide variety of complex architectures [1]. A grid of volumetric pixels (voxels) enables this advanced digital fabrication method, where each pixel's value represents either a 'soft' or 'hard' composite phase. This point-wise freedom allows incorporating complex bioinspired paradigms (e.g., functional gradients [2,3], multi-hierarchical organisation [3,4]) within designs. Nevertheless, computational modelling of large structures may be computationally expensive. Therefore, we developed a machine learning-based framework to homogenise the non-linear mechanical behaviour of voxel-based structures and tested it on different bioinspired designs.

We designed different sets of particle-reinforced composites with various reinforcement ratios. Then, we manufactured tensile tests samples of these structures with an Objet350 Connex3 printer (Stratasys® Ltd.). The test results and respective reinforcement ratios helped us generate a machine learning-based homogenisation model of any composite's non-linear mechanical behaviour. We performed validations with a subset of the designs using commercial FEM software (Abaqus v.6.14). Finally, we tested the model's performance by downscaling several bioinspired structures' resolution and comparing their mechanical behaviour.

The homogenisations accurately predicted the hyperelastic to elastoplastic transition observed in bitmap structures. We were also able to estimate both elastic and plastic regions for any composite's reinforcement ratio. Downscaling of structures with functional gradients and structures with features of low aspect ratios such as helixes was possible. Nevertheless, decreasing the resolution of fibre-like features showed a significant difference in property estimation. Future work will focus on developing algorithms that consider the unit-cell's overall orientation and its anisotropic properties.

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Abstract **#1402**

MERGING CARTILAGINOUS SPHEROIDS WITH MELT ELECTROWRITTEN MESHES FOR ENGINEERING BONE FORMING BIO-MEMBRANES

Gabriella Nilsson Hall[P][1,2], Angela Pastore[1], Amit Chandrakar[3], Katrina Moisley[4], Matei Cirstea[4], Liesbet Geris[1,2,5], Lorenzo Moroni[6], Paul Wieringa[6], Frank P. Luyten[1,2], Ioannis Papantoniou[1,7]

[1] *Prometheus, Div. of Skeletal Tissue Engineering, KU Leuven, Belgium*

[2] *Skeletal Biology and Engineering Research Center, KU Leuven, Belgium*

[3] *Department of Complex Tissue Regeneration, Maastricht University, The Netherlands*

[4] *The Electrospinning Company Ltd, England*

[5] *Biomechanics Research Unit, Université de Liège, Belgium*

[6] *Department of Complex Tissue Regeneration, Maastricht University, The NetherlandsDepartment of Complex Tissue Regeneration, Maastricht University, The Netherlands*

[7] *Institute of Chemical Engineering Sciences, Foundation for Research and Technology – FORTH, Patras, Greece*

Corresponding author's email: gabriella.nilssonHall@kuleuven.be

Progenitor cells from the periosteum are major contributors in fracture healing through involvement in the formation of a cartilaginous fracture callus. Recently, differentiated microspheroids assembled into scaffold-free constructs were shown to effectively heal murine long bone defects [1]. In this work, cartilaginous microspheroids were combined with designed melt electrowritten meshes to create a bioengineered callus-like membrane which resulted in bone formation through endochondral ossification.

Melt electrowritten polycaprolactone (PCL) meshes were tailored to contain pores (116 ± 28 µm) designed to allow efficient seeding of microspheroids (180 ± 15 µm). PCL meshes were coated with gelatin whereafter microspheroids attached and spread onto the mesh. After 14 days in chondrogenic media, gene expression analysis demonstrated up-regulation of chondrogenic (SOX9) and prehypertrophic (IHH) gene markers. To assess the bone forming capacity of the "living membranes" (day 14), they were implanted ectopically with meshes-only as control. No mineralization was detected in mesh-only explants but bone and mineralized cartilage was detected in all "living membranes" (25 ± 4 % MV/TV). These data demonstrated differentiation towards prehypertrophic chondrocytes in vitro and that the PCL scaffold did not notably hinder bone formation in vivo.

The high versatility of this biofabrication approach lies in the possibility to tailor the scaffolds to dimensions corresponding to robust biological building blocks, in this case microspheroids. We believe that these strategies will be instrumental in the development of designed living tissues with predictive clinical results upon implantation.

Keywords

Endochondral ossification; microtissue

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Abstract **#1403**

A NOVEL BIOREACTOR FOR MECHANOBIOLOGICAL STUDY OF ENGINEERED HEART VALVE TISSUE UNDER PHYSIOLOGICAL AORTIC HEMODYNAMIC CONDITIONS

Zhaoyang Zhong[P][1], Klaus Kreuels[2], Nadine Nottrodt[3], Arnold Gillner[2,3], Petra Mela[1]

[1] *Chair of Medical Materials and Implants MMI, Technical University of Munich*

[2] *Chair for Laser Technology LLT, RWTH Aachen University*

[3] *Fraunhofer Institute for Laser Technology ILT*

Corresponding author's email: zhaoyang.zhong@tum.de

The dynamic conditioning of in vitro tissue-engineered heart valves (TEHVs) is essential to promote synthesis and organization of the extracellular matrix and, ultimately, valve's functionality. To quantify the impact of the individual stimuli (i.e. cyclic stretching, pressure and shear stress) on the maturation of TEHVs, a novel bioreactor was developed in which engineered heart valve tissues can be conditioned under independently controlled mechanical stimuli. Four rectangular tissue specimens (20 x 10 x 1 mm) can be conditioned simultaneously in individual chambers with independent medium circulation. Computational fluid dynamic (CFD) was performed to support the design of the chamber to meet the requirement of creating laminar flow in the region of interest of the tissue specimen and a shear stress up to 71 dynes/cm2. Steady or pulsatile flow (max. average flow rate 2.3 l/min) can be generated, where the amplitude and frequency of pulsatile flow in each chamber can be independently controlled. Furthermore, axial tensile strain up to 25% can be applied to the tissues and physiological aortic pressure (80-120 mmHg) can be reached. The presented bioreactor provides a robust tool for the mechanobiological investigation of engineered heart valve tissue under physiological aortic hemodynamic conditions.

Abstract **#1404**

DESIGN AND VALIDATION OF A LOW-COST, VERSATILE TISSUE-ON-CHIP PLATFORM FOR COMPARTMENTAL CULTURES

Lorenzo P. Coppadoro[1], Gianfranco B. Fiore[1], Monica Soncini[1], Chiara Foglieni[2], Maria Lombardi[2]

[1] *Politecnico di Milano*

[2] *IRCCS San Raffaele Scientific Institute, Milano*

Corresponding author's email: lorenzopietro.coppadoro@polimi.it

Despite organ-on-chip technologies are now widespread in academic research, translation to commercial products is limited, due to high costs for large scale manufacturing [1], [2].

We have developed and validated a novel system fabricated with low-cost thermoplastic materials, exploiting CO2 laser machining [3], in view of possible adaptation to conventional industrial processes. The platform allows to perform compartmentalized cultures and to monitor in real time the culture growth. It consists of a multilayered cartridge, where the biological sample is hosted and of a 12-well plate compatible static module in which the cartridge is inserted. The versatility of the platform enables to host microporous membranes on which seeding cells, scaffolds, thin tissue biopsies, and precision-cut tissue slices (PCTS)[4]. At the end of the culture, the cartridge can be retrieved by means of a controlled snap-crack mechanism (patent pending) and reused to perform other experiments or analyses. This feature breaks new ground in the field allowing to subject the same sample to different sequential conditionings in different devices recapitulating various pathophysiological microenvironments, and to maintain the morphological integrity for subsequent analysis. We have biologically validated the device with Caco-2 cells and EAhy-926 cells, poured on microporous, commercially available, polycarbonate membranes (Whatmann Nucleopore® 5 µm pore size and it4ip ipCELLCULTURE™ 3 µm pore size, respectively), in static conditions. Cells confluence and density (DAPI), differentiation (JAM for Caco-2, CD-31 for EAhy-926) and gene expression (p53 for Caco-2, VEGF-A for EAhy-926) have been evaluated, showing results comparable with Transwell inserts, our golden standard.

Keywords

bicompartmental; retrieval; laser machining

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Abstract **#1406**

DEVELOPMENT OF A NOVEL TECHNIQUE TO CREATE AN ANISOTROPIC HYBRID MESH FOR HERNIA REPAIR

Alessandra Grillo[P][1], Augustin Barna[1], Zargham Hyder[2], Vivek Mudera[1], Alvena Kureshi[1]

[1] *Centre for 3D Models of Health and Disease, Division of Surgery and Interventional Science, University College London, London, UK*

[2] *Hydermed Limited, London, UK*

Corresponding author's email: alessandra.grillo.18@ucl.ac.uk

Most common commercial meshes for repair of abdominal hernia are made of synthetic materials, much stronger and stiffer than native tissue, causing recurrence and pain (1). Biological meshes derived from animal tissue provide good biocompatibility but they lack mechanical strength and often fail during tissue regeneration (2). Hence, hybrid meshes have been developed to overcome these issues, combining the strength of synthetic materials and the biocompatibility of biological components. Moreover, surgical meshes are isotropic while the fascial tissue mostly involved in herniation, displays anisotropy with highly aligned collagen fibres (3). This study aims to develop a hybrid mesh combining the benefits of synthetic and biological materials but also offers a unique feature of structural anisotropy to mimic the structure of native tissue. Hybrid constructs were fabricated by embedding a synthetic mesh into a standard collagen type I hydrogel. We developed a novel technique to create anisotropy within the gel which simultaneously combines horizontal shear flow to align the collagen fibres and the principles of the established RAFT technique to increase collagen density to tissue levels (4). The resulting collagen construct showed significant alignment of its fibres in the direction of shear flow. Importantly, this alignment is generated without the use of cells so the method has potential to be used in the fabrication of a novel mesh. Our next step is to demonstrate that this method can be used to generate an aligned collagen component that can be combined with the synthetic mesh to create our unique aligned hybrid mesh.

Keywords

Hybrid mesh; Hernia repair; Tissue engineering

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Abstract **#1407**

DECELLULARISED PLEURAL PATCHES FOR PROLONGED ALVEOLAR AIR LEAKS

Trisha Vikranth$_P$[1], Tina Dale[1], Nicholas Forsyth[1]

[1] *Keele University*

Corresponding author's email: t.vikranth@keele.ac.uk

Prolonged alveolar air leaks (PALs) as post-surgical complications to lung resections at an incidence of 26%, is a source of patient morbidity. The gold standard approach to managing PALs is conservative, through extended duration of chest tube drainage, often associated with thoracic pain, pulmonary and pleural infections, and extended length of hospital stays.

Aim:

Develop a tissue-engineered alternative with decellularised porcine pleural membranes (DPPM) as bioactive sealants, reinforcing conventional surgical procedures to seal air leaks, reducing the severity of PALs. The distinction is in its role as bioactive extracellular matrix scaffolds comparable in biochemical and mechanical attributes to native PPM, stimulating endogenous regeneration of lung tissue. Decellularisation ensures retention of native microarchitecture and composition of the membrane with reduced immune intolerance through removal of cellular antigens.

Methods:

PPMs were decellularised and characterised using histology, nuclear DNA staining (DAPI), membrane thickness estimation, and tensile testing.

Results:

H&E staining showed absence of purple stained nuclei in the DPPM, quantitatively confirmed with a significant difference in DAPI stained nuclei between DPPM and native PPM samples ($p < 0.0001$). Membrane thickness of DPPM (218 ± 68 µm) vs native (145.3 ± 33.1µm) were comparable ($p > 0.05$). Tensile testing estimated Young's modulus of DPPM (804±670kPa) vs native (828.4±177kPa) with no significant difference.

Conclusion:

Histological and mechanical studies indicate an efficient strategy to derive DPPM, with the removal of nuclear and cellular antigens and minimal disruption to native structural and mechanical properties. This represents the first step towards a clinical alternative to managing PALs.

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Abstract **#1408**

OPTIMIZATION OF 3D CANCER CELL CULTURE CONDITIONS BY APPLICATION OF CHEMICAL ENGINEERING PRINCIPLES

Mia Radonjic[1,2], Jelena Petrovic[P][1,2], Milena Milivojevic[3], Milena Stevanovic[3], Jasmina Stojkovska[1,2], Bojana Obradovic[1]

[1] *Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

[2] *Innovation Center of the Faculty of Technology and Metallurgy, Belgrade, Serbia*

[3] *Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*

Corresponding author's email: jelenapetrovic.co@gmail.com

Cancer cell immobilization in polymer hydrogels serving as extracellular matrices and cultivation in perfusion bioreactors that provide appropriate chemical signals, efficient mass transfer and hydrodynamic shear stresses is a promising strategy for development of physiologically relevant tumor models. In this work, perfusion cultures of 2 cancer cell types (C6 rat glioma and embryonal carcinoma NT2/D1 cells) immobilized in alginate microgels were established, while static cultures served as controls. Continuous perfusion had different effects on the cultured cells inducing enhanced proliferation of the glioma cells immobilized in microfibers (8x10^6 cell/ml), while reducing the viability of the NT2/D1 cells immobilized in microbeads (1x10^6 cell/ml). In order to elucidate the observed effects, chemical engineering principles were applied to assess mass transfer and hydrodynamic conditions. The second Fick's law was solved analytically while the diffusion-advection-reaction equation was solved numerically to model mass transport in the static and bioreactor cultures, respectively. Moreover, Reynolds numbers, pressure drops and shear stresses in bioreactor cultures were calculated for assessment of flow regime and hydrodynamic conditions. The modeling results have indicated that oxygen transport is diffusion-controlled through the alginate hydrogel, while medium perfusion improves mass transfer of larger compounds having smaller diffusion coefficients (~10^(-13) m^2/s), which possibly stimulated glioma cell proliferation. On the other hand, the obtained shear stress (~50 mPa) in the perfused packed bed of microbeads was above physiological levels, which provided the explanation of the poor NT2/D1 cell survival. This study stresses the importance of multidisciplinary approach in addressing such multifactorial diseases as cancer.

Abstract **#1409**

THERAPEUTIC POTENTIAL OF NAVITOCLAX IN AGE RELATED CARDIOVASCULAR DISEASES

Anna Walaszczyk$_P$[1], Emily Dookun[1], Rachael Redgrave[1], Pawel Palmowski[1], Simon Tual-Chalot[1], Eleanor Gill[2], Andrew Owens[1], David Grieve[2], Ioakim Spyridopoulos[1], Michael Taggart[1], Helen M. Arthur[1], João F. Passos[3], Gavin D Richardson[1]

[1] *Newcastle University, Newcastle upon Tyne, UK*

[2] *Queen`s University Belfast, UK*

[3] *Mayo Clinic, Rochester, MN, USA*

Corresponding author's email: anna.walaszczyk@newcastle.ac.uk

Cardiovascular disease (CVD) is the leading cause of death in individuals over 60 years old. Ageing is associated with decrease in myocardial function and a poorer prognosis following of myocardial infarction (MI). We have demonstrated that oxidative stress induced myocardial senescence contributes to both of these observations. In aged mice, prophylactic treatment with the senolytics drug navitoclax reduces senescence and the expression of SASP associated proteins, resulting in attenuated maladaptive myocardial remodelling and improved survival and functional outcome following MI. Furthermore, MI directly induces senescence and the SASP, as a result of increased oxidative stress, which are detrimental to outcome. In young animals, navitoclax treatment following MI with reperfusion improved left ventricular function, increased myocardial vascularisation, and decreased scar size. Proteomics revealed that elimination of senescent cells attenuated biological processes associated with maladaptive remodelling including fibrosis and inflammation. Cytokine array demonstrated navitoclax reduced expression of proinflammatory, profibrotic and anti-angiogenic cytokines, including interferon gamma-induced protein-10, TGF-β3, interleukin-11, interleukin-16 and fractalkine. Together our studies provide proof-of-concept evidence that cellular senescence and the proinflammatory SASP promote myocardial remodelling in multiple disease settings impairing heart function. Subsequently, senolytic treatment represents a potential novel therapeutic avenue to improve patient outcome for these CVDs.

Abstract **#1410**

SELF-ASSEMBLED DEOXYRIBONUCLEIC ACID HYDROGEL FOR TUNABLE DELIVERY OF MULTIPLE THERAPEUTIC AGENTS AGAINST MULTIDRUG RESISTANT CANCER

Liang-Hsin Chen[P][1], Tzu-Wei Wang[1]

[1] *Department of Materials Science and Engineering, National Tsing Hua University*

Corresponding author's email: sally003@gmail.com

Multidrug resistance (MDR) has been considered as a main impediment to cancer chemotherapy. In order to overcome drug resistance and inhibit cancer cells viability simultaneously, it is essential to develop a drug delivery system able to carry multiple therapeutic agents. Injectable hydrogel has been widely used in this aspect due to its in situ gelation property and the ability to encapsulate different bioactive molecules. In this study, tetra-armed PEG is crosslinked by complementary DNA sequences to form the network structure of hydrogel system with an MMP-2 cleavable peptide motif in between. Additionally, laponite nanoclay is added to finely tune the gelation and mechanical properties of the hydrogel. Due to specific hydrogen bonds formation between nucleobase pairing, the hydrogel is injectable and self-healable. MDR-targeted siRNAs including Pgp and Bcl-2 siRNAs, were encapsulated in the hydrogel to enhance therapeutic efficacy by overcoming MDR. In this hydrogel, both nucleic acid sequence and laponite nanoclay can be tailor-designed as delivery agents for anti-cancer drug, DOX. The two delivery agents attribute to sequential release of DOX, releasing DOX both before and after the effect of MDR siRNA. Moreover, the MMP-2 cleavable peptide between tetra-armed PEG and DNA deliberately controls hydrogel degradation; thus, resulting in the release of therapeutic cargo. With the tailored design of the hydrogel, we develop a drug delivery system by carrying multiple therapeutic molecules simultaneously to achieve synergistic effect and spatial-temporal control release for cancer therapy.

Keywords

Injectable hydrogel; Multi-drug resistance; RNA interference

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Abstract **#1411**

CALCIUM CONTAINING MESOPOROUS NANOPARTICLES FOR DRUG DELIVERY APPLICATIONS

Eleana Kontonasaki[P][1], Georgia Pouroutzidou[2], Maria Lazaridou[3], Evi Christodoulou[3], Ioannis Tsamesidis[1], Anna Theocharidou[1], Konstantinos M. Paraskevopoulos[2], Dimitrios Bikiaris[3]

[1] Department of Dentistry, Aristotle University of Thessaloniki, Greece

[2] Department of Physics, Aristotle University of Thessaloniki, Greece

[3] Department of Chemistry, Aristotle University of Thessaloniki, Greece

Corresponding author's email: kont@dent.auth.gr

Silica-based mesoporous nanocarriers (MSNs) have received widespread interest due to their ordered structure and high specific surface area, which allow fine control of drug loading and release kinetics1,2. Ca-containing MSNs are promising bioactive mesoporous drug carriers, as they can release Ca and Si ions that stimulate osteogenic differentiation of stem cells3. Moxifloxacin is an antibiotic with broad antimicrobial activity against aerobic and anaerobic bacteria, which could be applied locally for bacterial elimination in cases of periodontal or peri-implant desease4. The aim of this study was the evaluation of different Ca-doped MSNs in terms of moxifloxacin loading/release profiles, biocompatibility and hemolytic activity. The synthesis of MSNs was performed by the sol-gel method5. Their drug loading and release profiles were assessed with High Performance Liquid Chromatography (HPLC) and their cell viability with the MTT assay on human periodontal ligament cells (hPDLCs). Their hemolytic activity was evaluated in contact with human red blood cells at various concentrations. The loading capacity of Ca-doped MSNs (21-38%) was higher than pure silica (15%), revealing the beneficial effect of Ca. Prolonged release of moxifloxacin was apparent even after eight days. They presented limited hemolysis and no cytotoxicity, while promoting the mitochondrial activity and proliferation of hPDLCs.

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme “Human Resources Development, Education and Lifelong Learning” in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

Keywords

Calcium doped mesoporous nanoparticles; Moxifloxacin; Hemolytic activity

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Abstract **#1412**

EXTRACELLULAR MATRIX HYDROGELS FOR 3D BIOPRINTING LUNG RESIDENT MESENCHYMAL STROMAL CELLS

Jorge Otero$_P$[1], Bryan Falcones[1], Hector Sanz[1], Esther Marhuenda[1,2], Irene Mendizabal[1], Ignacio Cabrera[1], Isaac Almendros[1,2,3], Daniel Navajas[1,2,4], Ramon Farre[1,2,3]

[1] *University of Barcelona*

[2] *CIBER Respiratory Diseases*

[3] *IDIBAPS*

[4] *IBEC*

Corresponding author's email: jorge.otero@ub.edu

INTRODUCTION: One of the main hypotheses in mesenchymal stromal cells (MSCs) research is that the microenvironment determines the way that cells behave. Our aim is to investigate how bioprinting and 3D culturing lung resident MSCs in porcine lung-derived extracellular matrix (ECM) hydrogels produce changes in cell behavior. METHODS: Rat primary lung resident MSCs were bioprinted and 3D cultured in porcine lung ECM hydrogels presenting a stiffness of 0.7kPa. After seven days of 3D culture, cells were harvested from the scaffolds. Cell adhesion and actin/paxillin staining tests were conducted with the harvested and control cells by seeding them onto specific well-plates for optical imaging and allowed to attach them to the plate for 2h. The expression of surface chemokine receptor CXCR4 was quantified by qRT-PCR. RESULTS: Compared with cells cultured in standard tissue culture plates, cells harvested from the lung ECM hydrogel scaffolds formed focal adhesions 2-fold longer. Moreover, 10-fold more cells were adhered to the substrate after 2h. Finally, the expression of CXCR4 chemokine receptor showed a more than 20-fold increase in the preconditioned cells. DISCUSSION: The data indicate that culturing lung MSCs in the ECM has major impact in their adhesion capacity and in the expression of one the main receptors involved in several relevant processes in vivo. Thus, lung ECM-derived hydrogels have the potential to be used as a scaffold to develop novel in vitro models to better understand mechanisms in MSCs.

Keywords

Hydrogel; Bioprinting; Mesenchymal stromal cells

Abstract **#1413**

SILK FIBROIN KNIT SCAFFOLD FOR INCREASED INFILTRATION DEPTH AND IMPROVED ELSTICITY IN TISSUE ENGINEERING APPLICATIONS

Fatemeh Ajalloueian[1], Sanaz Khademolqorani[2], Hossein Tavanai[2], Anja Boisen[1]

[1] *Department of Health Technology, Technical University of Denmark, Ørsted Plads, 2800, Kgs. Lyngby, Denmark*

[2] *Department of Textile Engineering, Isfahan University of Technology, Isfahan, 84156-83111, Iran*

Corresponding author's email: faaj@dtu.dk

3D scaffolds are in the center of attention for tissue engineering applications. So far, there has been continuous progress and competition in design and engineering of biomaterials as scaffold candidates for regenerative medicine[1]. Whilst biomaterials-engineering approaches have mostly focused on a few mechanisms (mostly chemical), achieving suitable tissue biomechanical function remains an important challenge[2]. Besides, there are concerns that sustainable tissue engineering may not be achievable with current approaches[3]. This calls for further profound studies, emphasizing the need to imitate the structural and mechanical properties of the target tissue. Conventional 3D scaffolds suffer from limited depth of infiltration for cells due to their low porosity and/or not meeting the biomechanics of the target tissue, especially for regeneration of load-bearing tissues. In this study, we have designed and fabricated a model of silk fibroin knit scaffold, which along with post-fabrication degumming, results in a more bulky and less open structure compared with conventional knit fabrics. Our scaffold shows outstanding cell-scaffold interaction including full 3D cell attachment, 360-degree cell coverage around individual filaments, and full-thickness cell infiltration depth. The optimized structure alleviates the need for the in-advance filling of the pores and provides users with full depth access to the knit structure for increased cell adhesion and infiltration. From a mechanical viewpoint, the scaffold shows high elasticity and recovery upon unloading (up to around 90% strain), thanks to its intermeshed loops. Overall, our SF weft-knitted construct represents appropriate characteristics for the regeneration of load bearing tissues.

Keywords

Tissue engineering; Silk fibroin knit scaffold; Elasticity

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Abstract **#1414**

RAC1 GTPASE REGULATES COMPRESSION-INDUCED ACTIN PROTRUSIONS OF MESENCHYMAL STEM CELLS IN 3D COLLAGEN MICRO-TISSUES

KWOK LIM Lam[P][1], Yu Hin Wong[1], Sing Yian Chew[2], Pui Barbara Chan[1]

[1] *Tissue Engineering Laboratory, Biomedical Engineering Program, Department of Mechanical Engineering, The University of Hong Kong*

[2] *School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459, Singapore*

Corresponding author's email: vklhku@gmail.com

Cells can transmit mechanical signals to their interior via sub-cellular organelles including mechano-sensors, signalling molecules and cytoskeletal proteins, which play pivotal roles in cellular activities such as migration and orientation. Our previous results discovered mechano-responsive, omni-directional and compression-induced actin protrusions (CAPs) in human mesenchymal stem cells (MSCs) encapsulated in 3D collagen micro-tissues, but the mechano-regulation of CAP formation is still unknown. Here, we investigate the roles of three major RhoGTPases on mediating the formation of CAPs via an alteration of actin cytoskeletal reorganization in MSCs. Cyclic compression loading markedly upregulated Rac1 (but not CDC42 and RhoA) gene expression and its activity, suggesting its critical roles on the regulation of CAP formation. Upon GTPase suppression by specific inhibitors during compression, only Rac1 activity was significantly repressed, associating with significantly reduced CAP formation. Silencing the upstream regulators, including Rac1, of these pathways dramatically disrupted actin protrusions and elongated cell morphology. Silencing cortactin (CTTN), a proximal effector of Rac1 pathway, induced a compensatory activation of Rac1 signalling and its related downstream proteins without CAP formation but compression loading partially restored CAPs. The dominating role of Rac1 signalling and corresponding expression of lamellipodial markers suggest that these CAPs are very likely to be lamellipodia formed in 3D configurations upon compression loading-based stimulation. This study delineates the mechanism of compression-induced cytoskeleton reorganization in MSCs, rationalizing mechanical loading regimes for functional tissue engineering.

Abstract **#1415**

IMPRESSIVE ACCELERATION OF BONE REGENERATION BY ALLOGENEIC DEVITAL SOFT CALLUS MIMETIC CONSTRUCTS

Debby Gawlitta[P][1], Alessia Longoni[1], Lizette Utomo[1], Abbie Robinson[1], Riccardo Levato[2], Antoine Rosenberg[1]

[1] *Dept. of Oral and Maxillofacial Surgery & Special Dental Care, University Medical Center Utrecht*

[2] *Dept. of Orthopaedics, University Medical Center Utrecht*

Corresponding author's email: d.gawlitta@umcutrecht.nl

Clinical implementation of endochondral bone regeneration (EBR) could be significantly accelerated by the use of allogeneic devitalized cartilaginous templates. Nevertheless, previous attempts involving devitalized cartilaginous constructs showed subpar bone regeneration compared to their living counterpart (1,2). The aim of this study was to develop devitalized cartilaginous scaffolds that could enhance EBR in vivo. Additionally, the influence on EBR of chondrogenic or hypertrophic differentiation prior to devitalization was evaluated.

Allogeneic mesenchymal stromal cells (MSCs) were embedded in collagen and chondrogenically or hypertrophically induced prior to devitalization. It was confirmed that the devitalization procedure did not affect matrix composition (total protein, glycosaminoglycan, collagen) or ALP activity. Devitalized and vital chondrogenic or hypertrophic constructs were implanted in a critical-size rat femur defect. New bone formation was measured by micro-CT (4, 8, and 12 weeks) and confirmed with histology at 12 weeks.

Full bridging of all defects was observed for the devital chondrogenic (DC) constructs, already after 4 weeks. After 12 weeks, bone volume observed in the DC implants was superior compared to their living counterparts (97.4±11.2mm3 versus 38±26 mm3) and to both hypertrophic groups. Histological analysis displayed remodeling into cortical and cancellous compartments, only in the DC group. No adverse immunological reactions against the allogeneic DC implants were observed.

Here, we present for the first time MSC-derived devitalized cartilage constructs that go beyond the state-of-the-art and outperform their living equivalent in reproducibility and rate of bone regeneration. Their allogeneic origin and devital state are highly attractive for off-the-shelf clinical application.

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Abstract **#1416**

COMPARATIVE ANALYSIS OF EXTRACELLULAR VESICLES (EVS) DERIVED FROM ADIPOSE TISSUE- AND BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

Cansu Gorgun[P][1,2], Maria Elisabetta Federica Palama[1], Daniele Reverberi[2], Roberta Tasso[1], Chiara Gentili[1]

[1] *Department of Experimental Medicine, University of Genova*

[2] *IRCCS Ospedale Policlinico San Martino, Genova, Italy*

Corresponding author's email: cansu.gorgun@edu.unige.it

Mesenchymal Stromal Cells (MSCs) are effective therapeutic agents enhancing the repair of injured tissues mostly through their paracrine activity. Although MSCs are found in virtually all organs, bone marrow (BM)-MSCs and adipose tissue (AD)-MSCs are most commonly used in clinical practice. Increasing evidences show that besides the secretion of soluble molecules, the release of extracellular vesicles (EVs) represents an alternative mechanism adopted by MSCs. Within this, we aimed to characterize EVs released by both human AD-MSCs and BM-MSCs to investigate their involvement as modulators of MSC paracrine effects. EVs have been isolated from the conditioned media of AD- and BM-MSCs by serial differential centrifugations in order to collect both medium-sized and small-sized EVs. EV morphology has been characterized by transmission electron microscopy (TEM), while their size and concentration by tunable resistive pulse sensing (TRPS). The expression of the tetraspanin family members was evaluated using a non-conventional flowcytometry approach. The role of both medium and small-sized EVs in the different phases of the endochondral ossification process has been evaluated by ex vivo mouse metatarsal culture model. A human protein cytokine array was used to define the profile of the EVs secreted by MSCs. Although EVs derived from AD and BM-MSCs present similar characteristics in terms of size, concentration and marker expression, they possess significant differences in their protein content that are reflected in their functional effects. Taken together, these results indicate that EVs released by AD- and BM-MSCs present functional differences which should be taken into consideration when planning EV-based therapies.

Keywords

Extracellular vesicles; Multipotent (mesenchymal) stem cells

Abstract **#1417**

FUNCTIONAL ANALYSIS OF THE SOFT PALATE OF THE RAT BY USING WIRELESS ELECTROMYOGRAPHY

Doris Rosero-Salazar$_P$[1], Paola Carvajal-Monroy[2], F.A.D.T.G (Frank) Wagener[1], J.W. (Hans) von den Hoff[1]

[1] *Department of Orthodontics and Craniofacial Biology, Radboud*

[2] *Department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics, Erasmus Medical Center, Rotterdam, the Netherlands*

Corresponding author's email: doris.roserosalazar@radboudumc.nl

Cleft lip and palate (CLP) is the most common orofacial birth defect with a prevalence of about 1:1000 newborns causing problems mainly for suctioning and later for speaking[1]. About 45% of patients with CLP have a cleft in the soft palate. After surgery, dysfunction persists in around 30% of cases due to fibrosis[2]. The soft palate is formed by five different skeletal muscles of which the major one is the levator veli palatini (LVP)[3]. Our group established a model for muscle regeneration consisting of a biopsy wound in the LVP of the rat that shows fibrosis and impaired soft palate regeneration[3]. Yet, functional analysis of the rat soft palate has never been done up to now. Therefore the aim of this study is to establish a model to analyze the function of the normal and the wounded soft palate. Through wireless telemetric electromyography (EMG) the muscle electrical activity was measured while the rat is eating, drinking or grooming. While EMG signals are taken, the behavior of the rats is being video-recorded. The EMG signals and the videos were compared to analyze the muscle activity during these behaviors. Parameters such as train duration and peak amplitude were analyzed. The train duration did not show significant differences before and after surgical wounding while the peak amplitude was lower after wounding that indicates the function is affected in the wounded soft palate. This model for functional analysis will be useful to measure the muscle activity during regeneration after the application of muscle constructs.

Keywords

method development; cleft palate; electromyography

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Abstract **#1418**

ONE-STEP ASSEMBLY OF ROBUST ALL-CELLULAR FIBERS

Mariana B. Oliveira[1], Tânia Salvador[1], Ana Rita Sousa[P][1], João F. Mano[1]

[1] Department of Chemistry. CICECO – Aveiro Institute of Materials. University of Aveiro. Campus Universitário de Santiago, 3810-193 Aveiro

Corresponding author's email: ar.sousa@ua.pt

Scaffold-free regenerative approaches have captivated the interest of clinical stakeholders due to promising results achieved by pioneering strategies. However, biomaterial-free structures are often assembled either as thin two-dimensional cell sheets or spherical microaggregates, both limited on their ability to be deposited in a free-form manner, and dependent on complex processes for macroscale 3D upscaling. The shape of a fiber favors architectural free form positioning, and may be adapted to textile or printing technologies.We recently reported the rapid one-step fabrication of centimeter-long cellular fibers made of human mesenchymal stem cells derived from the adipose tissue (hASCs) [1]. A hanging column device enabled cells to be pulled to the apex of a stable liquid column, resulting in cell aggregation. Living hASCs fibers showed high cell viability, and were non-toxic in an ex vivo chick embryo chorioallantoic membrane (CAM) model. Cells could also invade simplified in vitro ECM-mimetic hydrogel models, while keeping the secretion of pro-regenerative and immunomodulatory factors overtime. The malleable character of the hASCs fibers enabled injecting them through needles and using them as shape-adaptable fillers in model defects. Despite their easy handling, the application of cell fibers in complex surgical procedures or as implantable living devices in tissues continuously subjected to mechanical stimulation justifies the development of tougher structures. By adjusting cell medium composition during cell assembly, we expect to develop cellular fibers with increased robustness, along with unaltered biological function.Acknowledgement: This work was supported by the POCI, component FEDER, and national funds through FCT/MCTES, project PTDC/BTM-ORG/30770/2017.

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Abstract **#1419**

IDENTIFICATION OF NEW BIOLOGICAL MARKERS OF SARCOPENIA BY PROTEOMIC ANALYSIS OF SERUM

Yves Henrotin[P][1], Antoine Florin[2], Anne-Christine Hick[3], Bérénice Costes[4], Alain Labasse[3], Christelle Boileau[5], Lhote Korine[6]

[1] *Université de Liège, Liège, Belgique*

[2] *MusculoSKeletal Innovative Research Lab (mSKIL), Arthropôle Liège, Center for Interdisciplinary Research on Medicines (CIRM) Liège, ULiège, Institute of Pathology, CHU Sart-Tilman, 4000 Liège, Belgium*

[3] *Biotesting, Artialis SA, Liège, Belgique*

[4] *Clinical, Artialis SA, Liège, Belgique*

[5] *Preclinical, Artialis SA, Liège, Belgique*

[6] *Research and development, Artialis SA, Liège, Belgique*

Corresponding author's email: yhenrotin@uliege.be

Background

Sarcopenia is the progressive generalized loss of skeletal muscle mass, strength, and function which occurs with aging. This study was undertaken to identify new biomarkers of sarcopenia by proteomics analysis of female sera.

Methods

A case control study was set-up, for which 19 sarcopenic subjects and 20 control subjects, according to the European Working Group on Sarcopenia Older People criteria published in 2010 (EWGSOP1), were enrolled. All the subjects being at least 65-year old and in majority female. Biomarker screening was done by a comparative mass-spectrometry analysis. Protein expression levels between the two groups were compared. One of the identified biomarkers, cathepsin D was measured by immunoassay on the serum of the full sample set (n=39). Its diagnostic performance was evaluated with a Receiver Operating Characteristic curve (ROC curve).

Results

Two biomarkers were identified: fructose-biphosphate aldolase A ($p \leq 0.05$) and cathepsin D ($p \leq 0.05$). The levels of all of them being higher in sarcopenic patients. It was confirmed by immunoassay that Cathepsin D levels in serum was significantly higher in the sarcopenic group of patients ($p = 0.038$). An inverse correlation (-0.385) was observed between cathepsin D levels in serum and gait speed. The area under the ROC curve measurement (AUC= 0.696) demonstrated that cathepsin D levels could discriminate between sarcopenic and non-sarcopenic subjects. A predictive model including cathepsin D, age and Body Mass Index was established to improve the diagnostic performance (AUC = 0.908).

Conclusions

Cathepsin D has been identified as a diagnostic biomarker of sarcopenia.

Abstract **#1420**

DISSECTING FIBROSIS IN HUMAN ANNULUS FIBROSUS AFTER INTERVERTEBRAL DISC HERNIATION

Ana Luísa Castro$_P$[1,2,3], Cláudia Ribeiro-Machado[1,2], Carla Oliveira[1,2,4], Paulo Pereira[1,2,5], Rui Vaz[1,2,6], Graciosa Quelhas Teixeira[7], Cornelia Neidlinger-Wilke[7], Mário Adolfo Barbosa[1,2,3], Raquel Madureira Gonçalves[1,2,3]

[1] *I3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal*

[2] *INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal*

[3] *ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal*

[4] *ESS - Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal*

[5] *Hospital CUF, Porto, Portugal*

[6] *Serviço de Neurocirurgia, Centro Hospitalar S.João, Porto, Portugal*

[7] *Institute of Orthopaedic Research and Biomechanics, Trauma Research Centre, Ulm University, Ulm, Germany*

Corresponding author's email: luisa.castro@i3s.up.pt

Intervertebral disc(IVD) herniation is the leading cause of spine surgery, in which failure of annulus fibrosus(AF) allows nucleus pulposus(NP) extrusion. During degeneration/herniation, IVD extracellular matrix(ECM) is remodeled, undergoing fibrosis, but this process is still under investigated, namely in the AF.

Samples (n=39) of human AF(hAF) collected from patients who underwent surgery for lumbar IVD and controls (n=6) of hAF from adolescent scoliosis patients were processed for histological analysis. ECM ultrastructure was evaluated by transmission electron microscopy(TEM), while biochemical content (sulphated glycosaminoglycans(sGAG), collagen 1(Col1)), fibronectin(FN)) and fibrotic markers (alpha-smooth muscle actin(αSMA+) and matrix metalloproteinase-12(MMP12)) were analyzed by histology, as well as collagen fibers distribution by Picrosirius Red. Uni-/Multivariate regression analysis was conducted to identify any association between ECM content/fibrotic markers with aging or IVD herniation progression.

For herniated patients, no association with Pfirrmann grade was found for the fibrotic markers. With aging, sGAG and Col1 decrease, while αSMA+ cells increase in young adults (22-40 age) and senior patients (>65). Independently of the age, the AF of patients with IVD herniation revealed structural changes, decrease in sGAG and Col1 content and increase of FN and αSMA+ cells with herniation progression (no significant interaction with age was found,$p<0.05$). Moreover, MMP12 and macrophages(CD68+) did not exhibit differences with aging or herniation progression.

hAF ECM content and fibrosis markers reveal differences with aging and herniation progression, that might be associated with an increase in αSMA+ cells, but not macrophages or MMP12. These findings reveal new insights about AF failure in IVD herniation.

Abstract **#1422**

VESSEL-ON-A CHIP FOR THE STUDY OF VASCULAR CALCIFICATION

Wilhelm Jahnen-Dechent$_P$[1], Andrea Büscher[1], Aaron Morgan[1], Jan Ritter[2], Ulrich Steinseifer[2]

[1] *Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University Hospital, Aachen, Germany*

[2] *Department of Cardiovascular Engineering, Institute of Applied Medical Engineering, Helmholtz Institute Aachen, RWTH Aachen University, Aachen, Germany*

Corresponding author's email: willi.jahnen@rwth-aachen.de

Cardiovascular disease (CVD) accounts for nearly one third of mortality in the aging population. Mortality is even higher in patients with chronic kidney disease (CKD). Phosphate retention in CKD is a major driver of endothelial damage, cardiovascular morbidity and mortality. Vascular calcifications in CKD patients are mainly located in the tunica media of a vessel, yet it is the endothelial cells (EC) that are especially vulnerable to phosphate-associated toxicity. Indeed, endothelial dysfunction is one of the earliest manifestations of vascular disease in CKD and other vasculopathies. To date however, the cellular cross-talk between EC and vascular smooth muscle cells (vSMC) have not been systematically studied in cell cultures, because suitable models are missing.

Cell monocultures fail to reflect the complex calcification pathology in the cardiovascular system. Cell culture medium is usually static and without flow. Cellular cross-talk as well as barriers between cell layers are missing and calcification media are unphysiologically supersaturated. We aimed to analyze vascular wall calcification using a more physiological dual-cell model. We developed a vessel-on-a-chip comprising endothelial cells cultured under flow next to smooth muscles cells cultured in a separate compartment without flow, while retaining the ability for cellular cross-talk. We employed established supersaturated simulated body fluids as well as protein-mineral complexes / calciprotein particles (CPP) as the calcification agent. The chip is suitable for live imaging and for testing biomimetic barrier materials separating the cells. At the endothelial cell side, we reached flow rates that produce around 10 dyn/cm2 shear stress simulating arterial blood flow.

Abstract **#1423**

COMPARATIVE STUDY OF DECELLULARIZATION METHODS FOR PORCINE SKELETAL MUSCLE

Charlot Philips$_P$[1], Lisanne Terrie[1], Lieven Thorrez[1]

[1] *Tissue Engineering Lab, Dept. of Development and Regeneration, KU Leuven, Belgium*

Corresponding author's email: charlot.philips@kuleuven.be

Skeletal muscle is of high clinical importance due to its role in metabolism and movement. A wide range of causes for muscle injury can have a debilitating effect on a person's life. However, current treatment strategies for volumetric muscle loss are limited to transfer of autologous muscle tissue, which is associated with donor site morbidity and incomplete functional recovery.

The development of acellular matrices through decellularization offers a promising approach to create scaffolds which retain the complex hierarchical structure of skeletal muscle tissue. Moreover, the preservation of the extracellular matrix (ECM) components could enhance the regeneration process. Challenges remain in finding the optimal balance between removal of cellular content without causing significant damage to the ECM and in the translation of effective decellularization protocols to skeletal muscle tissues of clinically relevant dimensions.

In this study, we compare different decellularization protocols to obtain acellular matrices of porcine skeletal muscle tissue with dimensions of 2 x 0.5 x 0.5 cm. Either the detergents Triton X-100, sodium deoxycholate or sodium dodecyl sulfate were employed, alone or in combination with enzymes such as trypsin and DNase I. We present both qualitative and quantitative data on the content of DNA, collagen and glycosaminoglycans. Furthermore, mechanical analysis reveals differences in viscoelastic properties of decellularized constructs.

We conclude that different combinations of decellularizing agents result in a different decellularization efficiency. However, it is feasible to achieve an acceptable decellularization of porcine skeletal muscle tissue with the proposed dimensions, which opens perspectives for further upscaling towards larger scaffolds.

Abstract **#1424**

IMMUNE PRINTS OF DEVITALIZED CARTILAGE GRAFTS CORRELATE WITH OUTCOME OF BONE FORMATION IN IMMUNO-DEFICIENT AND COMPETENT MICE.

Sujeethkumar Prithiviraj[P][1,2], Alejandro Garcia Garcia[1,2], Ani Grigoryan[1,2], Steven Dupard[1,2], Deepak B Raina[3,4], Magnus Tägil[3,4], Paul E Bourgine[1,2]

[1] *Laboratory for Cell, Tissue and Organ engineering, Lund stem cell center, Lund University, Sweden.*

[2] *Wallenberg Centre for Molecular Medicine at Lund University, Sweden.*

[3] *Department of Orthopedics, Lund University, Sweden.*

[4] *Clinical and Experimental bone healing.*

Corresponding author's email: sujeethkumar.prithiviraj.3684@med.lu.se

Introduction: Bone formation is a complex process. Understanding the associated cascade of cellular events would help the design of grafting material capable of inducing effective regeneration. Immunodeficient mice (IC) are standard for assessing the regenerative capacity of bone-grafts, despite their lower preclinical relevance. Here, we aim at investigating the impact of the immune cells on the bone formation capacity of our graft, in both immunocompetent (IC) and ID mouse models.

Method: Our graft consists of in vitro engineered cartilage tissue produced by human mesenchymal stromal cell (hMSCs) lines. This cartilage is subsequently devitalized by apoptosis induction, resulting in a cell-free graft aiming at instructing bone formation by endochondral ossification. The devitalized cartilage is implanted subcutaneously in the back of IC and ID animals for a maximum of 12 weeks. Recruitment of immune cells is investigated at day-3/7/10 post-implantation.

Results: Following in vivo implantation, a complete remodeling into bone was achieved in ID, whereas only minor calcification was observed in implants retrieved from IC mice. We further established a correlation between early immune cell recruitment (“immune prints”) and outcome of bone formation in ID and IC settings.

Conclusion and discussion: Our study illustrates the significance of the initial immune response during bone formation, especially macrophage recruitment and polarization. Ultimately, compiling such immune prints may not only be essential for assessing bone grafts immunogenicity, but also towards tuning their composition to activate/inactivate key immune pathways and ensure effective bone formation in clinical setting.

Keywords: Bone remodeling, Immunology.

Abstract **#1425**

INVESTIGATING LOCAL RELEASE OF SIMVASTATIN FROM A MINIMALLY INVASIVE THERMORESPONSIVE HYDROGEL TO ENHANCE OSTEOGENESIS

Christopher R. Simpson[P][1], Silvia Paiva[1,2], Helena M. Kelly[1,3], Ciara M. Murphy[1,2]

[1] *Tissue Engineering Research Group, Royal College of Surgeons Ireland, Dublin, Ireland*

[2] *Advanced Materials and Bioengineering Research (AMBER) Centre, CRANN Institute, Trinity College Dublin, Ireland*

[3] *School of Pharmacy and Biological Sciences, Royal College of Surgeons, Dublin, Ireland*

Corresponding author's email: christophersimpson@rcsi.com

Currently, efforts to reduce osteoporotic fracture risk include systemic anabolic treatments to promote bone formation or anti-catabolic therapeutics to prevent bone resorption, but these do not sufficiently repair and restore bone structure and function[1]. However, local delivery strategies of these therapies has yielded promising results boosting local bone repair with potential to mitigate osteoporotic fracture risk at clinically relevant sites[2,3]. We are interested in simvastatin (SVT) as a novel anabolic treatment to augment bone repair[4]. The aim of this project is two-fold: 1) Develop a thermoresponsive methylcellulose-collagen hydroxyapatite hydrogel that offers the potential to fill complex geometries found in osteoporotic bone, and provide a surrogate extracellular matrix to promote cellular infiltration and proliferation. 2) Investigate the release and osteogenic potential of SVT from the developed hydrogels. To date we have shown that the hydrogels are easily extruded from various needle gauges due to shear thinning properties at room temperature, undergo sol-gel transition upon approaching 37°C, resist degradation over 4 weeks when gelated and can support MC3T3-E1 (pre-osteoblast) cell viability and proliferation. These results demonstrate the suitability of the hydrogel for minimally invasive applications and site retention. The challenge remains to evaluate the osteogenic potential of this biomaterial and thereafter improve this by functionalizing with currently licensed anabolic or anti-catabolic therapies. Therefore, we further aim to harness the anabolic effects of simvastatin (SVT) to support osteoblast viability, facilitate osteogenesis and encourage mineralization. In parallel, the effect of SVT loaded hydrogel on osteoclast differentiation and bone resorptive activity will be determined.

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Abstract **#1427**

NOVEL METHOD FOR FAST 3D BIOPRINTING OF CLINICALLY RELEVANT TUBULAR STRUCTURES

Fernanda C. Bombaldi de Souza$_P$[1], Renata F. Bombaldi de Souza[1], Tácia T.A. Liguori[1], Leonardo O.P. Longo[1], Emerson G. Moretto[1], Gabriel R. Liguori[1]

[1] *TissueLabs SAGL*

Corresponding author's email: fernanda@tissuelabs.com

INTRODUCTION: Tubular structures (TS) are present in blood vessels, the urinary and respiratory tracts, and the gastrointestinal system. In the body, these structures are found mostly in the millimetric (>1mm) or centimetric (>10mm) scale. Still, coaxial bioprinting techniques currently available focus largely on producing micrometric (<1mm) TS. To produce clinically relevant TS, approaches focused on layer-by-layer deposition of bioinks require long bioprinting time. We propose a new method for fast 3D bioprinting of clinically relevant TS. METHODS: We developed an adapter to be attached to the extrusion unit of a 3D bioprinter and redirect the flow from two syringes towards a single coaxial channel. The adapter can be customized to have the inner and outer diameters ranging from 1mm to 10mm (or more, if desired). We fabricated TS using an alginate-based bioink and a Pluronic F-127/CaCl2 fugitive/crosslinking hydrogel. TS were produced using the conventional layer-by-layer method for comparison. RESULTS: TS of 1.5/5.0mm and 3.0/9.5mm (inner/outer) diameter and tunable length were produced. The printing time using our technique is standardized at 2s/mm, irrespective of the construct size. Using the conventional method (1mm/s flow rate), the printing time for the 1.5/5.0mm construct was 240s/mm and 900s/mm for the 3.0/9.5mm construct. Our method was 120x faster for the small construct and 450x faster for the large construct. CONCLUSION: 3D TS can be fabricated hundreds of times faster using our novel method of coaxial bioprinting.

Abstract **#1428**

AIR-FOAMED HYDROGELS FOR HOMOGENOUS CELL VIABILITY IN LARGE CONSTRUCTS

Tânia Salvador$_P$[1], Mariana B. Oliveira[1], João F. Mano[1]

[1] *Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*

Corresponding author's email: mboliveira@ua.pt

The increasing research interest on injectable hydrogels in tissue engineering and regenerative medicine is related to their ability to be used as cell-laden injectable scaffolding materials and compatibility with minimally invasive surgical techniques. Despite these advantages, large-scale bulk hydrogels are reported to hinder molecule and gas diffusion over large distances. This disadvantage associated with the poor solubility of oxygen in aqueous liquids[1] has led to the development of strategies for the production of microporous hydrogels[2] or the inclusion of oxygen-generating species[3] to enhance cell survival in such structures. We recently reported the development of cell-laden injectable gelatin methacryloyl (GelMA) foams, produced through a straightforward method based on the injection of air into a GelMA solution through the pull/push motion of a syringe plunger[4]. After 14 days of cell culture, human adipose-derived mesenchymal stem cells (hASCs) were found throughout the foam's whole volume, while GelMA bulk hydrogels showed uneven cellular distribution (hollow core without cells). Additionally, hASCs present in bulk hydrogels stained for hypoxia-inducible factor 1-alpha (HIF-1α), while such behavior was not observed in GelMA hydrogel foams. Therefore, we hypothesize that the oxygen present in the foam's air pockets may be slowly released and used by the cells, while on bulk hydrogels cells seem to migrate towards an oxygen-rich surface. To conclude, these results indicate that GelMA hydrogel foams may be advantageous for tissue engineering applications, particularly when homogenous tissue deposition in constructs with relevant sizes is required.

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Abstract **#1430**

POMEGRANATE EXTRACT TO IMPROVE MUSCLE RECOVERY AFTER ANTERIOR CRUCIATE LIGAMENT PLASTY? RESULTS OF AN EXPLORATORY, RANDOMIZED AND DOUBLE-BLIND STUDY

Yves Henrotin[P][1], Jean-Pierre Delcour[2], Jean-Louis Croisier[3], Antoine Florin[4], Thibaut Neutelings[5], Anne-Christine Hick[6]

[1] *University of Liège, Liège, Belgium*

[2] *Orthopedic surgery, C.H. Bois de l'Abbaye, Seraing, Belgium*

[3] *Physiotherapy and functional rehabilitation, University of Liège, Sart Tilman campus, Liège, Belgium*

[4] *MusculoSKeletal Innovative Research Lab (mSKIL), Arthropôle Liège, Center for Interdisciplinary Research on Medicines (CIRM) Liège, ULiège, Institute of Pathology, CHU Sart-Tilman, 4000 Liège, Belgium*

[5] *Preclinical, Artialis SA, Liège, Belgium*

[6] *Biotesting, Artialis SA, Liège, Belgium*

Corresponding author's email: yhenrotin@uliege.be

Introduction

This exploratory, monocentric, placebo-controlled, double-blind study aimed to study the effects of a Pomegranate extract (PE) on muscle recovery after anterior cruciate ligament plasty.

Patients and methods

One week after surgery, twenty-eight men received two food bars per day containing either PE/L-leucine/creatinine/vitamin D3/protein (n=14) or a placebo (n=14) for 12 weeks. The parameters studied were: pain at rest during the last 24 hours (Visual Analogue Scale (VAS)), disease activity assessed by the patient (AMP-EVA), physical performance by the "International Knee Documentation Committee (IKDC)" index, the peak torque of quadriceps and hamstrings using an isokinetic dynamometer and body composition by bio-impedancemetry (BIODY XPERT ZM®).

Results

There was no significant difference between the groups in pain, disease activity evaluated by the patient, physical performance (IKDC) and parameters of isokinetic exploration before and after 12 weeks of treatment. The lean mass decreased after surgery in the placebo group, while it remained stable in the PE group. However, the difference between groups was not significantly different. The lean mass compared to baseline was significantly higher in the PE group than in the placebo group after 2 (p=0.004) and 14 weeks of treatment (p=0.006). Finally, PE did not significantly alter basal metabolism compared to placebo. Compared to reference values, basal metabolism was lower in the PE group than in placebo group after two weeks.

Conclusion

Pomegranate extract had no significant effect on algo-functional status immediately after surgery for anterior cruciate ligament plasty but improve body composition by limiting the reduction in lean mass.

Abstract **#1431**

HYBRID DECM/POLYSACCHARIDE BIOINKS WITH TUNABLE RHEOLOGICAL PROPERTIES FOR 3D BIOPRINTING

Renata F. Bombaldi de Souza$_P$[1], Fernanda C. Bombaldi de Souza[1], Tácia T. A. Liguori[1], Leonardo O. P. Longo[1], Emerson G. Moretto[1], Gabriel R. Liguori[1]

[1] *TissueLabs SAGL*

Corresponding author's email: renata@tissuelabs.com

INTRODUCTION: Polysaccharides are extensively used in bioink formulations for extrusion-based 3D bioprinting due to their biocompatibility and processability. However, their low bioactivity is a major drawback. Tissue-specific decellularized extracellular matrices (dECMs), in turn, contain biochemical components that provide the ideal microenvironment for cellular attachment, proliferation, migration and differentiation. Herein, we combined the viscoelastic behavior of xanthan gum and the fast cross-linking ability of alginate with the unique bioactivity of dECM to achieve a bioink with good printability and superior biological performance. METHODS: Alginate-xanthan solution (1:3) was mixed with a lung dECM hydrogel at 75:25 (A), 85:15 (B) and 90:10 (C) weight ratios. Bioink viscosity at increasing shear rates and viscosity recovery were determined using a rotational viscometer. Printability was assessed by filament fusion and collapse tests using a 3D bioprinting system. RESULTS: The bioinks presented a shear-thinning behavior and their viscosity decreased with increasing dECM proportion. At high shear rates, viscosity of C was 1.7 and 1.1 times higher than A and B, respectively. Viscosity recovery was lower for bioink A than for B and C, reaching up to 98.5%. The printability is higher for C, with minimum material spreading. Filament of bioink C did not collapse between two consecutive pillars of up to 5 mm distance, while the filaments of B collapsed at 3 mm and of A at 2 mm. CONCLUSION: Bioinks with a range of rheological properties and good printability were obtained from the combination of polysaccharides and dECM.

Keywords

decellularized extracellular matrix; polysaccharide; rheological properties

Abstract **#1432**

VITREOUS HUMOR AS INSTRUCTIVE BIOMATERIAL TO SUPPORT MESENCHYMAL STEM CELL HYPERTROPHY AND ENDOCHONDRAL OSSIFICATION

Debby Gawlitta[P][1], Alessia Longoni[1], Iris Pennings[1], Gabriella Lindberg[2], Tim Woodfield[2], Khoon Lim[2], Antoine Rosenberg[1]

[1] Department of Oral and Maxillofacial Surgery & Special Dental Care, University Medical Center Utrecht, Utrecht, the Netherlands

[2] Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, Department of Orthopaedic Surgery and Musculoskeletal Medicine, Centre for Bioengineering & Nanomedicine, University of Otago Christchurch, Christchurch, New Zealand

Corresponding author's email: d.gawlitta@umcutrecht.nl

An essential step in endochondral bone regeneration (EBR) is to ensure an appropriate chondrogenic differentiation state prior to in vivo implantation. Vitreous humor (VH), the gelatinous mass between the lens and the retina, has emerged as a cell-instructive substrate that promotes mesenchymal stromal cell (MSC) chondrogenesis (1). The aim of this study was to establish the suitability of VH to support MSC progression towards a hypertrophic phenotype in vitro and EBR in vivo.

MSCs embedded in VH hydrogels were stimulated to achieve an early (chondrogenic medium only) or late (chondrogenic + hypertrophic medium) hypertrophic phenotype. After 31 days, the presence of chondrogenic (glycosaminoglycan and collagen type II) and hypertrophic (collagen type X and mineralization) markers was evaluated. Chondrogenic or hypertrophic MSC-laden VH hydrogels were implanted in a subcutaneous rat model for 8 weeks. Bone formation was evaluated by micro-CT (2,4,6 and 8 weeks), fluorochrome injections (2,4 and 6 weeks) and histological analysis (8 weeks).

VH supported a reproducible differentiation, as chondrogenic and hypertrophic markers were present in all samples from 3 different MSC donors. Once implanted, chondrogenic and hypertrophic MSC-laden VH constructs induced comparable neo-bone formation. However, a later onset of bone formation, larger portion of non-remodeled cartilage and smaller bone marrow cavities were observed in the hypertrophic group, compared to the chondrogenic one.

VH hydrogels effectively support MSC chondrogenesis and hypertrophy in vitro. Irrespectively of the culture conditions prior to implantation, EBR was observed in vivo. This indicates that VH is an ideal biomaterial for EBR-based regenerative approaches.

Keywords

Vitreous humor; Endochondral; Natural hydrogels

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Abstract **#1433**

A FIBRINOGEN-BASED BIOINK FOR SKIN TISSUE ENGINEERING

Aida Cavallo[P][1,2], Marianna Buscemi[2], Ilenia Foffa[2], Tamer Al Kayal[2], Giorgio Soldani[2], Paola Losi[2]

[1] *Sant'Anna School of Advanced Study, Institute of Life Sciences, Pisa, Italy*

[2] *Institute of Clinical Physiology, National Research Council, Massa, Italy*

Corresponding author's email: aida.cavallo@ifc.cnr.it

The 3D bioprinting enables to prepare skin tissue engineered grafts for non-healing chronic wounds or skin defects leading to effect on patients quality life1. Fibrin structural composition and binding to cells highly determine wound healing process2.

The aim is to develop a fibrinogen-based bioink with addition of alginate for skin constructs printing followed by fibrin cross-linking using a thrombin–calcium chloride (CaCl2) solution.

Several concentrations of bovine fibrinogen and sodium alginate were dissolved in deionized water. These solutions were semi-crosslinked by mixing with 100mM CaCl2 solutions at varying volumetric ratios. The crosslinking-solution has bovine thrombin (50 UT/ml) dissolved in 50mM CaCl2 solution.

The solutions printability and biocompatibility were characterized by: homogeneity test; spreading ratio; SEM; compression test; water uptake; degradation and cell viability/proliferation test on L929 mouse fibroblasts using LIVE/DEAD and XTT assay at 0-7 days3,4.

The bioink with the lowest spreading ratio and water up-take values, slower degradation rate and better cell proliferation was mixed with human dermal fibroblasts (6x106 cells/ml) and human epidermal keratinocytes (8x106 cells/ml) to print a bilayered skin constructs using a 25G conical nozzle, 12mm/s of speed and 10KPa of extrusion pressure. The bio-printed constructs were cultured up to 14 days and evaluated through histological analysis by H&E staining and Masson's trichrome.

The developed bioink is composed by semi-crosslinked alginate (25:9 as volumetric ratio) and 30 mg/ml of fibrinogen. The bio-printed constructs showed homogeneous viable cells distribution and collagen deposition.

These preliminary results showed that the fibrinogen-based bioink developed enabling cells proliferation post-printing and tissue maturation.

Keywords

Bioprinting

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Abstract **#1435**

EFFECT OF THE BUFFER ON THE BUILDUP AND STABILITY OF TANNIC ACID/COLLAGEN MULTILAYER FILMS APPLIED AS ANTIBACTERIAL COATINGS

Muhammad Haseeb IQBALP[1], André Schroder[1], Halima Kerdjoudj[2,3], Christian Njel[4], Bernard Senger[5,6], Vincent Ball[5,6], Florent Meyer[5,6], Fouzia Boulmedais[1]

[1] Université de Strasbourg, Centre National de la Recherche Scientifique, Institut Charles Sadron, UPR 22, 23 rue du Loess, BP 84047, 67034 Strasbourg Cedex 2, France

[2] Université de Reims Champagne Ardenne, EA 4691, Biomatériaux et Inflammation en Site Osseux (BIOS), SFR CAP Santé (FED4231), 51100 Reims, France

[3] UFR d'Odontologie, Université de Reims Champagne Ardenne, 51100 Reims, France

[4] Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein- Leopoldshafen, Germany

[5] Institut National de la Santé et de la Recherche Médicale, UMR_S 1121, 11 rue Humann, 67085 Strasbourg Cedex, France

[6] Université de Strasbourg, Faculté de Chirurgie Dentaire, 8 rue Sainte Elisabeth, 67000 Strasbourg, France

Corresponding author's email: haseeb.iqbal@ics-cnrs.unistra.fr

Development of multifunctional surfaces has drawn great attention over the years. Surface properties like antibacterial activity and biocompatibility are highly desired to allow tissue integration of biomedical implants.[1] Medical devices can undergo nosocomial infections caused by bacterial attachment and their growth into undesired biofilm formation, making them exceedingly resistant to antibiotic treatments.[2] In Europe, 1 patient out of 20 develops a nosocomial infection during hospitalization, posing a huge burden on the healthcare facilities and economy.[3] Prevention of the early attachment and proliferation of bacteria has gained popularity and led to the development of antibacterial coatings. Among several routes to modify surfaces, layer-by-layer (LbL) is a versatile method to design biocompatible and antibacterial coatings to prevent infections. Tannic acid (TA), a natural polyphenol, is known to inhibit the growth of several bacteria. Herein, we developed TA/collagen (TA/COL) LbL films built in acetate or citrate buffers at pH 4. Surprisingly, the used buffer impacts not only the physicochemical but also the antibacterial properties of the films. When incubated in physiological conditions, both types of TA/COL films released approximately same amount of TA depending on the last layer and showed an antibacterial effect against Staphylococcus aureus only for citrate-built films, without cytotoxicity towards human gingival fibroblasts. ITC and XPS demonstrate that the complexation strengths between TA and COL are different in the presence of both buffers that modifies the amount of TA immobilized. This work constitutes an important step toward the use of polyphenols as an antibacterial agent when incorporated in LbL films.[4]

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Abstract **#1436**

PORE SIZE OF SCAFFOLDS ENABLES DISTINCT MECHANORESPONSIVE BEHAVIOUR OF OSTEOARTHRITIC CHONDROCYTES

Yu Zhao[P][1,2], Zhifeng You[3], Dan Xing[4], JiaoJiao Li[5], Jianhao Lin[4], Yanan Du[3]

[1] *Arthritis Clinic and Research Center, Peking University People's Hospital, Peking University, Beijing, China*

[2] *The Second Hospital of ShanXi Medical University*

[3] *Department of Biomedical Engineering, School of Medicine, Tsinghua University, Beijing, China*

[4] *Arthritis Clinic and Research Center, Peking University People's Hospital, Peking University, Beijing, ChinaPeople's Hospital, Peking University,Beijing,China*

[5] *Faculty of Medicine and Health, Kolling Institute, University of Sydney, St. Leonards, Australia.*

Corresponding author's email: zhaoyu20806@163.com

Osteoarthritis (OA), there is more pronounced cartilage damage in the medial compartment ('lesion zone') than the lateral compartment ('remote zone'). This study fills a gap in the literature by conducting a systematic comparison of cartilage and chondrocyte characteristics from these two zones. It also investigates whether chondrocytes from the different zones respond distinctly to changes in the physical and mechanical microenvironment using three-dimensional porous scaffolds by changing stiffness and pore size. Cartilage was harvested from patients with end-stage varus knee OA. Cartilage from the lesion and remote zones were compared through histological and biomechanical assessments, and through proteomic and gene transcription analyses of chondrocytes. Gelatin scaffolds with varied pore sizes and stiffness were used to investigate in vitro microenvironmental regulation of chondrocytes from the two zones. Cartilage from the lesion and remote zones differed significantly ($p<0.05$) in histological and biomechanical characteristics, as well as phenotype, protein, and gene expression of chondrocytes. Chondrocytes from both zones were sensitive to changes in the structural and mechanical properties of gelatin scaffolds. Of interest, although all chondrocytes better retained chondrocyte phenotype in stiffer scaffolds, those from the lesion and remote zones, respectively, preferred scaffolds with larger and smaller pores. Distinct variations exist in cartilage and chondrocyte characteristics in the lesion and remote zones of knee OA. Cells in these two zones respond differently to variations in the physical and mechanical microenvironment. Understanding and manipulating these differences will facilitate the development of more efficient and precise diagnostic and therapeutic approaches for knee OA.

Abstract **#1437**

GENERATION AND FUNCTIONAL CHARACTERIZATION OF A 3D ENGINEERED PERFUSED HEART TUBE AS IMPLANTABLE BIOLOGICAL CARDIAC ASSIST DEVICE

Ina Gruh[P][1,2,3], Andres Hilfiker[1,2,3], Andreas Martens[4,2], Robert Zweigerdt[1,2,3], Birgit Andree[1,2,3], Monika Szepes[1,2,3], Nils Benecke[1,2], Wiebke Triebert[1,2,3], Thomas Aper[4,2], Axel Haverich[4,2,3], Ulrich Martin[1,2,3]

[1] *Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO)*

[2] *Hannover Medical School, Hannover, Germany*

[3] *REBIRTH Research Center for Translational Regenerative Medicine*

[4] *Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG)*

Corresponding author's email: gruh.ina@mh-hannover.de

Background and Aim - Due to its structural and functional complexity, the heart imposes immense challenges on engineering a biological replacement, therefore we propose a simpler biological assist device for future support or replacement of failing human hearts. Our goal is to generate a 3D-perfused heart tube (3D-Heart-2B) based on human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).

Methods – 3D-Heart-2B is designed as a modular system based on the following components and strategies: Highly compacted fibrin-based myocardial-like tubes generated from iPSC-CMs for contractile function and iPSC-derived vascular cells for de novo vessel formation; novel strategies for instant perfusion; decellularized heart valves to direct medium and ultimately blood flow in bioreactors and mock circulation models; in vitro maturation and testing; surgical strategies in animal models for in vivo testing.

Results and Conclusions – We have generated highly compacted fibrin-based tubes containing up to 200 x 106 iPSC-CMs with a length of 10 cm, a diameter of 5-6 mm and an initial wall thickness of 0.6 mm. Cellular distribution and vitality in the 3D-Heart-2B construct was assessed by live cell imaging and immunohistological staining. Contractile function was demonstrated after 4 and 5 weeks of cultivation (d28 and d35). Implantation of the 3D-Heart-2B tube in an ex vivo perfused pig heart mounted in the Organ Care System (OCS) as a bypass graft demonstrated suturability but so far limited mechanical stability. Following scale-up to clinically relevant dimensions, the 3D-Heart-2B construct will fulfill key criteria of an implantable biological cardiac assist device for failing hearts.

Abstract **#1439**

IN SITU TISSUE ENGINEERING VASCULAR ACCESS GRAFTS IN A LARGE ANIMAL MODEL

Paul Besseling[P][1,2], Wojciech Szymczyk[3], Martin Teraa[2], Raechel Toorop[2], Dan Jing Wu[3], Joost Fledderus[1], Carlijn Bouten[3], Gert Jan de Borst[2], Patricia Dankers[3], Marianne Verhaar[1]

[1] *Department of Nephrology and Hypertension, Regenerative Medicine Centre, University Medical Centre Utrecht*

[2] *Department of Vascular Surgery, University Medical Centre Utrecht*

[3] *Department of Biomedical Engineering, and Institute for Complex Molecular Systems, Eindhoven University of Technology*

Corresponding author's email: P.J.Besseling@UMCUtrecht.nl

Vascular access is considered the Achilles' heel of hemodialysis, resulting in frequent and recurring inverventions1. Autologous options are limited by pre-existing disease, previous interventions, and/or unsuitable vessels. Synthetic alternatives are susceptible to infection, intimal-hyperplasia, and/or thrombosis2. We aimed to create an off-the-shelf, porous, biodegradable, self-healing vascular access graft by means of in situ tissue engineering that in vivo gradually transforms into a living vascular graft. Our biodegradable supramolecular elastomeric materials-platform enables us to modulate the mechanical properties and biofunctionalise our graft to maximise (intended)cellular integration. We investigated the remodelling and functional capacity of these arterio-venous(AV)-grafts and the contribution of SDF1α bio-functionalisation in a large animal model.

Electrospun, porous, biodegradable, vascular scaffolds(6mm-diameter) made of supramolecular polymers, reinforced with a PCL 3D-printed coil to prevent kinking, were bilaterally implanted in goats(approx.65kg) as AV shunts, connecting the carotid artery and jugular vein. A subset was functionalised with SDF1α linked to the polymer material3. Patency was monitored by ultrasound. Grafts were explanted after 3months and analysed for mechanical properties, material breakdown, cellular infiltration, ECM deposition and tissue architecture.

After 3months 50% of the grafts remained patent, showed remodeling, and bore no signs of deformations. The synthetic material was resorbed and replaced by autologous tissue consisting of vascular cells and ECM. SDF1α functionalisation showed no effect. Our first implantations of an off-the-shelf, biodegradable, porous, non-kinking vascular scaffold in a goat model showed clear remodeling, gradually replacing the scaffold by autologous vascular tissue. This shows great promise for off-the-shelf in situ tissue engineered vascular access grafts.

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Abstract **#1440**

A THERMO-SENSITIVE SEMI-INTERPENETRATING CHITOSAN/PECTIN NETWORK FOR 3D CRC SPHEROID CULTURE

Giulia Morello$_P$[1,2], Alessandra Quarta[1], Antonio Gaballo[1], Alessandro Polini[1], Giuseppe Gigli[1,2], Lorenzo Moroni[1,3], Francesca Gervaso[1]

[1] CNR NANOTEC – Institute of Nanotechnology, c/o Campus Ecotekne, Via Monteroni, 73100 Lecce, Italy

[2] University of Salento, Department of Mathematics and Physics "E. De Giorgi" via Arnesano, 73100 Lecce, Italy

[3] Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, department of complex tissue regeneration, 6229ER, Maastricht, The Netherlands

Corresponding author's email: giulia.morello@unisalento.it

Organoid systems represent an alternative approach for studying human organism and modeling human tissues and diseases (1), allowing the development of targeted therapies for individual patients (2). In contrast to traditional 2D in vitro and animal models, organoid cultures provide a more careful study of cell-cell and cell-matrix interaction mechanisms (3). These systems could be a valid tool for studying tumor microenvironment by using 3D biomaterial such as hydrogels, which imitate the tissue microenvironment and facilitate patient tumor cultures. To this aim, we developed an innovative thermo-sensitive hydrogel based on naturally derived polymers such as chitosan (CH) and pectin (PEC), which weakly interact to form polyelectrolyte complex (4). We exploited the characteristic of CH to crosslink with β-glycerophosphate (BGP) to form a stable polymeric network incorporating PEC into the system, thus originating a semi-Interpenetrating Polymer Network (semi-IPN). The developed PEC/CH semi-IPN resulted thermoresponsive at 37°C, injectable at room temperature, suitable for cell-encapsulation (pH=7.4), stable in vitro up to 3 weeks, a super-absorbent material with highly interconnected porosity and permeable to small-large nutrients (3-5 to 70 KDa). Tunable mechanical properties (around 1-4 kPa) and permeability, could be reached by varying the CH/PEC content. Finally, the PEC/CH semi-IPN was successfully used to induce human colorectal cancer cell (HCT116) spheroids formation up to 45 days of culture. The CRC-like spheroids dimension and density were influenced by the PEC/CH semi-IPN stiffness and permeability. These encouraging results would allow the implementation of faithful 3D tumor models for the study and development of personalized oncological treatments.

Keywords

Natural Polymers; 3D Colorectal cancer (CRC) Tumor models; Thermo-sensitive hydrogel

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Abstract **#1441**

IDENTIFYING AND MONITORING OF EMERGING RISKS RELATED TO BIOMATERIALS USING THE RISK RADAR APPROACH: EXAMPLE OF THE EU-PROJECT PANBIORA

Somik Chakravarty[P][1], Aleksandar Jovanović[2], Peter Klimek[3], Marjan Jelic[1]

[1] *Steinbeis Advanced Risk Technologies GmbH (R-Tech), Stuttgart, Germany*

[2] *European Virtual Institute for Integrated Risk Management (EU-VRi), Stuttgart, Germany*

[3] *Section for Science of Complex Systems, CeMSIIS, Medical University of Vienna, Vienna, Austria*

Corresponding author's email: schakravarty@risk-technologies.com

Advances in tissue engineering and manufacturing technologies have led to the rapid production of hundreds of biomaterials. The informational deluge generated from sources such as scientific articles, news, social media, etc. have created a technological challenge related to identifying, monitoring, and communicating potential risks.

As part of the risk pre-assessment, horizon scanning can capture early notions that are potentially associated with emerging risk (ER) that may develop into fully born risks over time. Horizon scanners such as the Risk Radar, conceptualised in the EU project, iNTeg-Risk [1] offers an organised and formal process of gathering, analysing unstructured big-data and disseminating value-added information to support decision making.

The Radar uses a bipartite network of documents and words extracted from splitting each document to individual words using word bagging [2]. The document sources include openly accessible abstracts of scientific publications, blogs, news articles, etc. Two parameters, Relevance and Novelty are defined and measured. While the former quantifies how central the documents are positioned in the term-document network, the later calculates the number of different topics a given document covers and is related to its expected impact. The parameters are combined to form a composite indicator "Criticality" based on the EU JRC COIN methodology [3].

The Risk Radar allows identification and monitoring of ERs, along with interconnectedness and quantification of the systemic importance of ERs. The Radar is developed as a practical web-based tool and is further developed for early recognition of new, emerging risks in biomaterials for the EU project PANBioRA.

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Abstract **#1442**

HOW MATHEMATICS CAN SUPPORT REGENERATIVE MEDICINE IN TERMS OF SIMULATION & DATA PROCESSING

Patrick Mehmert[P][1,2], Mathieu Perennou[1,2], Thomas Reiher[1,2], Philip Sperling[3,2]

[1] *Simufact engineering GmbH*

[2] *Hexagon AB*

[3] *Volume Graphics GmbH*

Corresponding author's email: patrick.mehmert@simufact.de

Mathematics is used to describe, predict and optimize the world since milleniums. It has evolved from simple counting to complex topics beyond most of our imagination. The development of computers enabled the efficient use of numerics to solve problems where the effort was too high to be accomplished by pen and paper. A “natural” area of applying mathematics is technical engineering. But mathematics is universal so that using simulation in the fields of biology and medicine also got more and more common.

One application for the benefit of regenerative medicine can be using generative design. Designing artificial body parts can be based on the real loads and material strength thus delivering the optimal geometry and weight. Mimicking only the mechanical function of a body part can lead to unneccesary weight or too less strength and endurance. A new approach is presented where even non-simulation experts can quickly and reliably generate such parts.

These complex shapes can thankfully be produced by 3D printing nowadays. Nevertheless it is advisable to simulate manufacturing to predict and mitigate problems like distortion or failures. This enables a faster and less costly production at a higher quality. The current capabilities and limitations are presented.

Patients as well as implants are often examined by imaging technology like CT and MRT. The raw data obtained gets most of it meaning first after processing this data and making available all the possibilities of image evaluation. An overview on how mathematics powers the measurement data processing is given.

Abstract **#1443**

DEVELOPMENT OF A PIPE-LINE TO CREATE AND CHARACTERIZE CANINE AND FELINE ORGANDIES; LIVER AND PANCREAS AS EXAMPLES

Louis C. Penningp[1]

[1] *Utrecht University, Faculty of Veterinary Medicine*

Corresponding author's email: l.c.penning@uu.nl

Companion animals, like dogs and cats, can be instrumental in preclinical studies. However, both for preclinical and clinical studies, species differences must be taken into account in order to appreciate the true value of the measurements. To create a balance between in vivo studies with obvious animal welfare issues, and cell line measurements, with obvious physiological limitations, in vitro cultured mini-organ, so-called organoids, can provide a best-of-both-worlds situation.
After the creation of canine liver organoids, we developed feline liver organoids, and rapidly afterwards canine intestinal organoids, bovine liver organoids, porcine liver organoids, and canine pancreas organoids. All of these models had specific research questions to be answered. E.g. canine liver organoids were created as alternative for whole liver transplantation (pre-clinical model) (1,2), whereas feline liver organoids were used to screen for drugs affecting feline hepatic lipidosis (3,4).
The knowledge acquired during these 5 years of organoid technology in veterinary medicine has let to the development of a rapid method to create and characterize organoids from other organs.
The very recent development of canine pancreas organoids will be presented as an example how efficient this pipe-line is and (to be honest with you) the drawback of this technology and hurdles to expect will be described too.
The experience with organoid cultures clearly highlights the difference between species, even at in vitro culture level.

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Abstract **#1444**

PROTEOMIC CHARACTERISATION OF EQUINE PLATELET-RICH PLASMA FROM ADULT PERIPHERAL BLOOD

Aggie Turlo$_P$[1], Aibek Smagul[1], Peter Clegg[1], Mandy Peffers[1]

[1] *Department of Musculoskeletal and Ageing Science, Institute of Life Course and Medical Sciences, University of Liverpool, UK*

Corresponding author's email: a.turlo@liverpool.ac.uk

Platelet-rich plasma (PRP) is used extensively in human and veterinary medicine to enhance tissue repair, however, its mechanism of action remains unclear. This study aimed to comprehensively profile the proteome of equine PRP to provide insights on potential mechanisms of tissue healing and compare it with existing human data.

PRP was obtained through double centrifugation method [1] from blood samples collected from four adult healthy horses culled at a commercial abattoir (PLT 441 x 103/µl ± 187, WBC 17 x 103/µl ± 5). Liquid chromatography-tandem mass spectrometry was used for global proteomic profiling of PRP following high abundant protein depletion (ProteoMiner). Proteins were identified with a 1% false discovery rate and minimum of two unique peptides per protein. Equine protein identifications were converted to human orthologs for bioinformatic analysis.

238 proteins were identified across all samples. Core dataset including 193 proteins found only in 4/4 and 3/4 biological replicates was generated and functionally characterized by gene ontology terms. Enrichment analysis (STRING) indicated ‘extracellular region’ and ‘secretory granule’ as the main cellular components, ‘vesicle-mediated transport’ as biological process and ‘protein binding’ as molecular function. Searching against Vesiclepedia database showed that 76% of proteins were found in human extracellular vesicles. 67% of proteins were previously identified in human PRP proteome [2].

Extracellular vesicles and their cargo constitute a major part of equine PRP proteome and may be responsible for mediating biological effect of PRP. Equine PRP studies may be useful to inform human application of PRP due to similarities in protein composition.

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Abstract **#1445**

SYSTEMATIC STUDY OF THE PRECLINICAL TECHNOLOGY READINESS OF ORTHOPAEDIC GENE THERAPY AND OUTLOOK FOR CLINICAL TRANSLATION

Georg A Feichtinger[1], Piers Wilkinson[P][1,2], Ilya Bozo[3], Thomas Braxton[1,2], Elena Jones[4], Roman V Deev[5], Peter V Giannoudis[4,6]

[1] *Division of Oral Biology, School of Dentistry, University of Leeds, Leeds, UK*

[2] *CDT Tissue Engineering & Regenerative Medicine, Institute of Medical and Biological Engineering, University of Leeds, Leeds, UK*

[3] *Federal Medical Biophysical Center, FMBA of Russia, Moscow, Russia*

[4] *Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK*

[5] *Ryazan State Medical University, Ryazan, Russia*

[6] *Academic Department of Trauma and Orthopaedics, School of Medicine, University of Leeds, Leeds General Infirmary, Leeds, UK; NIHR Leeds Biomedical Research Centre, Chapel Allerton Hospital, Leeds, UK*

Corresponding author's email: mnpw@leeds.ac.uk

Bone defects and impaired healing of fractures are an increasing public health burden and represent an unmet clinical need. Gene therapy has been investigated as a promising approach to improve or augment bone healing with the potential to provide true functional regeneration. While large numbers of studies have been performed in vitro or in vivo in small animal models that support the use of gene therapy for bone repair, these systems do not recapitulate key features of a critical/complex fracture environment. An overview of preclinical studies in large animal models, ongoing clinical work and a technology readiness assessment (TRA) are presented to identify potential translational bottlenecks and provide recommendations to overcome them. The current state of orthopaedic gene therapy research in preclinical large animal models was systematically reviewed. Whilst the results and outlook in the current preclinical research literature are generally positive, inconsistent methodologies across studies were a limitation in addressing factors vital for translation and risk of bias with regards to study design was observed. Systematic analysis suggests that most preclinical approaches tested in large animals currently sit within technology readiness level (TRL) 4. A comprehensive overview of clinical studies and the status of translation is provided in the context of the regulatory environments in the EU, US and Russian Federation. This work aims to provide a robust basis and outlook for continued, standardised and translation-tailored development of gene therapy for orthopaedic indications and beyond in regenerative medicine.

Abstract **#1446**

CIRCADIAN ANGIOGENESIS IN A 3D SYSTEM

Valeria Mastrullo[1,2,3], Priyanka Gupta[2], Daan Van der Veen[3], Jonathan Johnston[3], Paolo Madeddu[4], Paola Campagnolo[P1], Eirini Velliou[2]

[1] Cardiovascular Section, Department of Biochemical Sciences, University of Surrey, Guildford GU2 7XH, UK.

[2] Bioprocess and Biochemical Engineering Group (BioProChem), Department of Chemical and Process Engineering, University of Surrey, Guildford GU2 7XH, UK.

[3] Chronobiology Section, Department of Biochemical Sciences, University of Surrey, Guildford GU2 7XH, UK.

[4] Experimental Cardiovascular Medicine, University of Bristol, Bristol Heart Institute, Bristol Royal Infirmary, Bristol BS2 8HW, UK.

Corresponding author's email: p.campagnolo@surrey.ac.uk

Introduction. Whereas healthy human adult vasculature is quiescent, the development of new capillaries from the existing vasculature (angiogenesis) constitutes a critical step for regenerative medicine and tissue engineering. Recent evidence suggests that circadian rhythms (CR) are involved vascular physiology, particularly angiogenesis, whilst the 3D-microenvironment of the vasculature is critical for the spatiotemporal maturation of capillaries and the regulation of endothelial cells (EC) and pericytes (PC). This study aims to evaluate the effect of circadian clock on angiogenesis in a 3D-environment.Methods. Tube formation was evaluated in a 3D-Matrigel assay. Highly-porous polyurethane (PU)-scaffolds, fabricated by Thermal-Induced Phase Separation [1], were surface modified with fibronectin/gelatin to mimic the extracellular matrix. Primary human EC and PC were cultured in PU-scaffolds either individually or co-cultured together. Stack images were acquired using confocal laser-scanning microscopy.Results. Multicellular Matrigel assay showed a clear influence of CR on the organisation and conformation of capillary-like structures, especially when EC and PC were both synchronised. Alongside our results showing PC circadian rhythmicity for the first time in vitro, these indicate a role for CR during angiogenesis. Multicolour confocal imaging revealed that EC and PC organise in vascular-like structures around pores in polymeric 3D-scaffolds over 2 weeks of culture. Discussion/Conclusions. This preliminary data provides the basis for future advanced study of circadian regulation of angiogenesis in 3D tissue-engineered scaffolds. This would offer a reliable in vitro platform for chronobiology applications, recapitulating the complex spatial distribution of cells in their natural environment, while maintaining the tuneable characteristics of a tissue-engineered microenvironment.

Keywords

Angiogenesis; Circadian

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Abstract **#1447**

MIMICKING HUMAN TYMPANIC MEMBRANE: THE SIGNIFICANCE OF GEOMETRY

Shivesh Anand$_P$[1], Thomas Stoppe[2], Mónica Lucena[1], Marcus Neudert[2], Serena Danti[3], Lorenzo Moroni[1], Carlos Mota[1]

[1] Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

[2] Ear Research Center Dresden, Department of Otorhinolaryngology, Head and Neck Surgery, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

[3] Department of Civil and Industrial Engineering, University of Pisa, Pisa, Italy

Corresponding author's email: s.anand@maastrichtuniversity.nl

The thin and concave tissue of tympanic membrane (TM) captures sound pressure waves from the environment and transforms them into mechanical motion [1]. The successful transmission of these acoustic vibrations is attributed to the intricate three-dimensional (3D) architecture of the TM, consisting of radially and circumferentially aligned collagen fibrils [2, 3]. In this work, we investigate the influence of this precise geometrical construction on its mechano-acoustic response by applying relevant computational and biofabrication approaches.

A Python script was developed for generating 3D models highlighting the key anatomical features of the human TM. Three test designs along with a plain control were chosen to decouple some of its dominant structural attributes. A dual-scale fabrication strategy combining electrospinning and additive manufacturing was carefully optimized to manufacture scaffolds within the dimensions of the native TM. Preliminary in silico modeling performed in COMSOL Multiphysics suggested a geometrical dependency of their mechanical and acoustical responses, where the presence of radially aligned fibers was observed to have a more prominent effect as compared to their circumferential counterparts. The experimental characterizations conducted using macro-indentation and laser Doppler vibrometry confirmed similar mechano-acoustic results. Finally, human mesenchymal stromal cells and human dermal fibroblasts cultured on the TM scaffolds demonstrated a desirable alignment and collagen deposition along the fabricated patterns.

This work synergizes theoretical and experimental approaches toward understanding the significance of geometry in tissue engineered TM scaffolds. Furthermore, with the optimized biofabrication strategies, a radical improvement has been achieved in the previously reported limits for manufacturing alloplastic TM replacements.

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Abstract **#1448**

COLONIES OF INDUCED PLURIPOTENT STEM CELLS SELF-ORGANIZE PROGRESSIVELY UNDER SPATIAL CONFINEMENT AND THIS PROCESS CONTINUES IN 3D AGGREGATES

Mohamed Hamdy Elsafi Mabrouk[P1], Roman Goetzke[1], Guilio Abgnale[1], Burcu Yesilyurt[1], Lucia Salz[1], Kira Zaevert[1], Zhiyao Ma[1], Marcelo Szymanski[1], Ronghui Li[2], Ivan Costa[2], Vivek Pachauri[3], Uwe Schnakenberg[3], Martin Zenke[1], Wolfgang Wagner[1]

[1] *Institute for Biomedical Engineering – Cell Biology, RWTH Aachen University Medical School, Aachen, Germany*

[2] *Institute for Computational Genomics, RWTH Aachen University Medical School, Aachen, Germany*

[3] *Institute of Materials in Electrical Engineering 1, RWTH Aachen University, Aachen, Germany*

Corresponding author's email: mmabrouk@ukaachen.de

Colonies of induced pluripotent stem cells (iPSCs) are heterogeneous and their subsets organize towards the outer rim. To gain better insight into this self-organization we used either an array of PDMS pillars or micro-contact printing (µCP) to geometrically confine the growth of iPSCs. Subsets with higher expression of the pluripotency markers OCT4, E-cadherin, and NANOG progressively segregated to the outer 50-70 µm of the colonies. After 5 days the self-organized colonies were analyzed with single-cell RNA-sequencing (scRNA-seq) and the OCT4high subpopulation revealed most prominent upregulation of NODAL and its inhibitor LEFTY. In fact, these proteins were also higher expressed at the outer rim, whereas intermittent disruption of calcium-dependent cell-cell interaction abrogated this self-organization and resulted in uniform expression of OCT4 and NODAL. After 5 to 7 days the self-organized iPSC colonies spontaneously detached from their µCP substrates to form three-dimensional (3D) aggregates. This new method facilitates robust generation of embryoid bodies (EBs) of controlled size, without enzymatic or mechanical treatment. The procedure could even be implemented on a semi-automatic liquid handling unit and the resulting EBs could be differentiated towards all three germ layers. Notably, early 3D aggregates revealed similar self-organization as observed in 2D colonies with marked upregulation of OCT4, E-cadherin, and NODAL at the outer layer. Furthermore, scRNA-seq demonstrated progressive upregulation of similar genes in the OCT4high subpopulation, particularly of LEFTY1, TDGF1, NODAL, and genes of the TGF-ß/SMAD signaling pathway. Our results demonstrate that the spatial self-organization of iPSC colonies continues at their transition into EBs.

Abstract **#1449**

MODULATION OF INFLAMMATION AND MACROPHAGE PHENOTYPE OF THE SYNOVIUM USING TAA IMPROVES MIGRATION OF MESENCHYMAL STROMAL CELLS

Marinus (Tim) Wesdorp[1], Serdar Capar[1], Kavitha Sivasubramaniyan[1], Yvonne Bastiaansen-Jenniskens[1], Roberto Narcisi[1], Gerjo van Osch[1,2,3]

[1] *Department of Orthopaedics, Erasmus MC, University Medical Center Rotterdam, the Netherlands*

[2] *Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology, the Netherlands*

[3] *Department of Otorhinolaryngology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands*

Corresponding author's email: m.wesdorp@erasmusmc.nl

Therapeutic solutions for osteochondral defects using an endogenous approach are emerging. Osteochondral defects are often accompanied by synovial inflammation. We investigated how synovial inflammation influences mesenchymal stromal cell(MSC) migration, and whether modulation of inflammation improves migration.

Osteoarthritic synovial tissue explants were cultured with/without 1µM triamcinolone acetonide(TAA) for 24h to obtain synovium-conditioned medium(SCM). The effect of 6 SCM donors on migration of passage 3 BMSCs was examined in Boyden chamber assays. Inflammation of the synovial explants was assessed with gene expression analysis and flow cytometry for macrophage subtypes. Human peripheral blood monocytes were stimulated with TNF-α/IFN-γ towards pro-inflammatory-, with IL-4 towards repair-, and with IL-10 towards anti-inflammatory macrophages. After polarization they were cultured with/without 1µM TAA for 24h, the conditioned medium was used to assess BMSC migration.

Modulation of synovium with TAA significantly decreased expression of TNFA, IL1B, and IL6, genes associated with inflammation, and increased gene expression of CD163, associated with anti-inflammatory macrophages. The percentage of CD14+/CD80+($p<0.001$) or CD14+/CD86+($p<0.001$) pro-inflammatory macrophages was lower, whereas the percentage of CD14+/CD163+ anti-inflammatory macrophages was higher($p<0.001$) in samples treated with TAA than without TAA. Modulation of synovium with TAA resulted in a 1.5-fold increase($p<0.01$) in migration of BMSCs. Moreover, BMSC migration increased 3.1-fold($p<0.001$) in response to medium conditioned by repair macrophages, and 2.3-fold($p=0.02$) by anti-inflammatory macrophages when these macrophages were exposed to TAA.

Decreased synovial inflammation increased BMSC migration. Modulation of inflammation and macrophage phenotype in synovium using TAA might be promising to enhance BMSC migration in osteochondral defect repair.

Abstract **#1450**

MECHANOBIOLOGICALLY MIMETIC MODEL SYSTEMS OF HEALTHY AND METASTATIC BONE

Syeda Masooma Naqvi[1], Vatsal Kumar[1], Laoise McNamara[1]

[1] *Mechanobiology and Medical Device Research Group, Biomedical Engineering, National University of Ireland Galway, Ireland*

Corresponding author's email: syeda.masoomanaqvi@nuigalway.ie

Mechanobiological cues are proposed to encourage tumor cell invasion [1, 2], however, the specific biomechanical cues that govern metastasis to bone are not understood. Although in vitro models have been developed to study bone metastasis [3, 4], these have not incorporated mechanical cues that occur in vivo. The objectives of this study were to (1) develop an engineered biomimetic 3D in vitro multicellular model system that replicates in vivo mechanical cues and (2) investigate the biophysical regulation of bone metastasis. Gelatin-nHA (Nano hydroxyapatite) encapsulated OCY454 osteocytes and MC3T3 preosteoblasts were layered and cultured in the presence of osteogenic factors for 21 days to allow mineralisation. Next, gelatin-nHA encapsulated RAW264.7 preosteoclasts (Healthy model), or RAW264.7 and 4T1 adenocarcinoma cells (Metastatic model), were added to the mineralised constructs. The constructs were mechanically stimulated (0.5Hz, 0.5% strain 1h/day) within a custom bioreactor and cultured without osteogenic factors for 3 and 7 days. Static controls were included. In the healthy model, osteoclast activity (CTSK positive multi-nucleated cells) was confirmed under static conditions at day 7. Mechanical stimulation reduced this osteoclast activity and maintained already deposited mineral. In the metastatic model, positive IL-6 staining increased under static conditions, when compared to healthy conditions. Interestingly, the addition of mechanical stimulation reduced IL-6 staining in the metastatic model relative to the healthy counterpart. These results confirm the establishment of an engineered biomimetic 3D in vitro multicellular model system with confirmed osteoblast, osteoclast and metastatic activity and thereby provides an advanced model for studying bone metastases.

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Abstract **#1451**

MASS SPECTROMETRY IMAGING FOR FRACTURE HEMATOMA ANALYSIS

Rald Groven[P][1,2], Jane Gruisen[2], Maxime Eveque-Mouroux[2,3], Sylvia Nauta[2,3], Martijn Poeze[2], Martijn van Griensven[1], Tiffany Porta-Siegel[3], Berta Cillero-Pastor[3], Taco Blokhuis[2]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Cell Biology-Inspired Tissue Engineering, Maastricht University, Maastricht, The Netherlands*

[2] *Department of Surgery, Maastricht University Medical Center +, Maastricht, The Netherlands*

[3] *Maastricht Multimodal Molecular Imaging (M4I) Institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, The Netherlands*

Corresponding author's email: r.groven@maastrichtuniversity.nl

Although fracture treatment improved over the last decades, a substantial part of all fractures shows delayed healing and complications such as non-union(1,2). Fracture healing is a complex process, involving cell-cell interactions, various cytokines and growth factors(1,3,4,6,8). Although the fracture hematoma (FH) is known to have a relevant role in this process, the exact mechanisms are poorly understood(1,2,3,5-7). To improve strategies in fracture treatment, regulatory pathways in fracture healing need to be investigated. Lipids are important structural components of the cell and function as messenger compounds, therefore this study initiates with lipid spectra analysis.

The aim of this study is to identify lipid spectra in FH over time with matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). MALDI-MSI enables analyses of lipids and their spatial distribution without prior knowledge, biological- or chemical labeling. Therefore, this method is more time-efficient and requires less tissue than conventional methods.

For this study, fourteen FH samples were surgically removed, snap frozen, sectioned, washed and analyzed at different timepoints after fracture injury (1-19 days). Principal component analysis and discriminant analyses identified three groups based on distinctive m/z values which were associated to FH age; early (1-3 days, n = 4), middle (6-10 days, n = 6) and late group (12-19 days, n = 4). This shows that MALDI-MSI is well capable of making clear distinctions in lipid spectra between the early, middle and late FH group. These findings warrant further research into FH analysis by means of MALDI-MSI and its possible clinical implications in fracture treatment.

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Abstract **#1453**

ENZYMATIC HIGH HYDROSTATIC PRESSURE FOR UTERINE TISSUE DECELLULARIZATION AND REGENERATION

Narintadaech Charoensombut$_P$[1], Dongzhe Wang[2], Kinyoshi Kawabata[2], Jeong Hyun Kim[3], Tsuyoshi Kimura[4], Akio Kishida[4], Takashi Ushida[2], Katsuko S. Furukawa[3,2]

[1] *Department of Bioengineering, University of Tokyo*

[2] *Department of Mechanical Engineering, University of Tokyo*

[3] *Department of Bioengineering, University of Tokyo*

[4] *Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University*

Corresponding author's email: ch.narintadeach@gmail.com

Infertility is the main obstacle to achieve pregnancy naturally for women. The current treatments for infertility can not be applied for women who are suffering from uterus malfunction. Regenerative medicine and tissue engineered organ is a promising solution, which scaffold plays an important role for tissue regeneration. In this research, we developed a combination method to fabricate decellularized scaffold by using enzyme and high hydrostatic pressure. The enzymatic high hydrostatic pressure method can fabricate uterine scaffold within 40 minutes. Results showed cells were removed from native uterine tissue while preserving the natural macro- and microstructure of extracellular matrix. For in vivo, scaffold showed tissue regeneration in murine model. Moreover, pregnancy test was successfully achieved. Thus, the enzymatic high hydrostatic pressure showed a promising solution for uterine tissue engineering.

Keywords

Uterus; Decellularised

Abstract **#1454**

BIOASSEMBLIES OF DISTINCT CARTILAGE MICROTISSUES TO ENGINEER SCAFFOLD-FREE OSTEOCHONDRAL IMPLANTS

Gabriella Nilsson Hall$_P$[1,2], Wai Long Tam[3], Leire Casas-Fraile[3,4], Liesbet Geris[1,3,5], Frank P. Luyten[1,3], Ioannis Papantoniou[1,6]

[1] *Prometheus Division of Skeletal Tissue Engineering, KU Leuven, O&N1, Herestraat 49, PB 813, 3000 Leuven, Belgium*

[2] *Skeletal Biology and Engineering Research Center, Department of Development and Regeneration, KU Leuven, O&N1, Herestraat 49, PB 813, 3000 Leuven, Belgium*

[3] *Skeletal Biology and Engineering Research Center, Department of Development and Regeneration, KU Leuven, O&N1, Leuven, Belgium*

[4] *Laboratory of Tissue Homeostasis and Disease, KU Leuven, O&N1, Herestraat 49, PB 813, Leuven 3000, Belgium*

[5] *Biomechanics Research Unit, Université de Liège, Belgium*

[6] *Institute of Chemical Engineering Sciences, Foundation for Research and Technology-Hellas, Stadiou 26504, Platani, Patras, Greece*

Corresponding author's email: gabriella.nilssonHall@kuleuven.be

Bioassembly of microtissues has the potential to meet the difficulties of creating complex osteochondral tissues. Microtissue bioassembly relies on the development and progressive fusion of the microtissue modules. In this study, we investigated in vitro microtissue fusion and demonstrated that bioassembled constructs preserved their spatial properties in vivo. This was shown for both human adult periosteum derived cells (hPDC) as well as human induced pluripotent stem cells (hiPSC) as cell source.

hPDC microspheroids were created in agarose microwells and differentiated towards the chondrogenic lineage. A hierarchical construct was formed by assembly of three hPDC-derived cartilaginous microtissue intermediates of progressive maturity from i) early day 7, ii) mature day 14 to iii) (pre)hypertrophic organoids day 21. Upon ectopic implantation in nude mice, the formation of a hybrid tissue with bone, mineralized cartilage and fibrocartilage was observed. To engineer more stable articular-like cartilage, hiPSC-derived hyaline cartilage microtissues (iCMT) were introduced in combination with the pre-hypertrophic cartilage organoids (day 21) to create dual structures. iCMT fused slower during dual assembly and were therefore incubated longer to allow formation of a stable construct. Both tissue zones revealed their biological potency upon ectopic implantation, corresponding to the pre-engineered zonal pattern.

The assembly of functional building blocks as presented in this work, opens possibilities for the production of complex tissue engineered implants by embedding zone-specific functionality through the use of properly pre-programmed living building blocks.

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Abstract **#1455**

ASSESSMENT OF THE NEUTROPHIL RESPONSE TO A PANEL OF HARD AND SOFT BIOMATERIALS; A NOVEL COMPREHENSIVE IN VITRO APPROACH

Marinus (Tim) Wesdorp[1,2], Andrea Schwab[1], Roberto Narcisi[2], David Eglin[1], Gerjo van Osch[2,3], Matteo D' Este[1]

[1] *AO Research Institute, Davos, Switzerland*

[2] *Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands*

[3] *Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology, the Netherlands*

Corresponding author's email: m.wesdorp@erasmusmc.nl

Neutrophils play a pivotal role in orchestrating the immune response to biomaterials, the onset and resolution of chronic inflammation and macrophage polarization. Neutrophil response can be described by: survival, neutrophil extracellular trap (NET) formation, and release of granules with immunoregulatory factors. We investigated the processes in human primary neutrophils exposed to different biomaterial surfaces.

Human primary neutrophils of five healthy donors were seeded at a concentration of 1.0*106/mL in wells coated with a panel of hard and soft biomaterials. Cell-survival and metabolic activity were determined after 1,3,5,7, and 24 hours. Formation of reactive oxygen species(ROS) and NET components myeloperoxidase and elastase were assessed after 3 hours. Secretion of inflammation-related factors was evaluated biochemically.

Neutrophil survival was 90-100% after 24 hours on the soft hydrogel surfaces, but was lower than 50% on tissue culture plastic(TCP) and polycaprolactone(PCL). Metabolic activity after 1, 3, 5, and 7 hours was 2-3-fold higher on TCP and PCL than on soft materials. MPO and elastase levels were 3-10-fold higher on TCP, PCL, and polyvinyl alcohol(PVA) surfaces than on hyaluronan-and collagen-containing hydrogels. In contrast, ROS production was higher on soft hydrogels than on TCP and PCL. MMP9 secretion was significantly higher on TCP, PVA and PCL surfaces than on hyaluronan-and collagen-containing hydrogels.

Biomaterials deriving from natural proteins resulted in higher levels of ROS, whereas neutrophils respond with more NET formation on synthetic materials. Stiffness and composition of substrates play a role in neutrophils responses suggesting the ability to be potential characteristics to modulate the inflammatory response.

Abstract **#1456**

ELECTROPROCESSING SOY SCAFFOLDS WITH BENCHTOP AND HANDHELD DEVICES FOR REGENERATIVE MEDICINE APPLICATIONS

Yah-el Har-el[P][1,2], Jonathan A. Gerstenhaber[1], Azadeh Timnak[1], Sogol Moaiyed Baharlou[1], Peter I. Lelkes[1,2]

[1] *Temple University Department of Bioengineering*

[2] *Temple Institute for Regenerative Medicine and Engineering (TIME)*

Corresponding author's email: yahel@temple.edu

Plant-derived biomaterials hold promise as regenerative materials for wound healing applications. Using electrospinning as a platform, we developed and tested soy-based wound dressings that entail a biomimetic nanofibrous network ideal for cell interaction, growth, and differentiation.[1] Using a pig model, we previously demonstrated that electrospun soy protein isolate (SPI) can enhance the healing in both the wound bed and the epithelium.[2] Similar results have also been found when comparing electrospun SPI side-by-side to a commercial acellular matrix, Oasis.[3] In order to improve cellular infiltration into the scaffolds, we modified the electrospinning process creating a gradient porous fibrous (GPF) scaffold, with pore sizes ranging from 7.8 ± 2.5 µm on the small pore side to 58.0 ± 23.6 µm on the large pore side.[4] In order to avoid toxic solvents, we began using a water-soluble soy protein isolate (WSsoy) and combined electrospinning and electroblowing for a methodology termed “electroprocessing”. Electroprocessed WSsoy scaffolds exhibit smaller diameter fibers (200 ± 20 nm)[5] and are formed more rapidly than by electrospinning alone. Both GPF and WSsoy scaffolds showed enhanced wound healing in a rat model. As electroprocessing is less sensitive to the distance from the needle to the target, we developed a hand-held device for applying electroprocessed biomaterials directly to a wound, with improved healing both in terms of gross wound healing and at the deeper, cellular architecture level. Taken together, electroprocessing is a promising technology for advancing wound healing and the field of regenerative medicine.

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Abstract **#1458**

POLISH BIOBANKING NETWORK – THE POSSIBILITY OF LINKING SCIENCE, TECHNOLOGY, AND BUSINESS

Angelika Paluch[1,2], Anna Chróścicka[P][1,2,3]

[1] *Laboratory for Cell Research and Application, Center for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland*

[2] *BBMRI.pl Consortium*

[3] *Department of Histology and Embryology, Center for Biostructure Research, Medical University of Warsaw, Warsaw, Poland*

Corresponding author's email: anna.chroscicka@wum.edu.pl

The biobank's tasks are not only collecting, processing and storage of biological samples and connected with them information, but also developing technology and commercial solutions.In 2016, Poland as BBMRI.pl Consortium joined the European biobank's network BBMRI-ERIC. The Consortium consists of highly specialized units, which professionals are working on the development of broadly understood biobanking in Poland. The main task of BBMRI.pl is to create Polish Biobanking Network (PBN) which aim is to join Polish biobanks and optimize their work. This is supposed to lead to the advancement of science, medicine, pharmacy, and related fields.There are many biobanking entities in Poland. PBN has already joined 50 units. Most of them declare specialized, clinical, or mixed (specialized-population) character. There are also some population biobanks in Poland. The material collected in biobanks is various types, mainly of human origin. Due to its diversity, it can be used in many preclinical studies. The quality standards that biobanks care about and advancement of used technology ensure the credibility of the biobanked material and obtaining reliable results of research in which it was used.Biobank's resources are often associated with rare, genetic, or oncological diseases. Thanks to the use of biobanked material, the acceleration and reduction of research costs at the preclinical stage is observed. That is why biobank can be named as an institution of a new type, combining the interests of independent entities.Key words: biobank, Polish Biobanking Network, BBMRI.pl This work was supported by MNSW, grant DIR/WK/2017/2018/01-1

Abstract **#1460**

A 3D IN VITRO PERIPHERAL NERVE MODEL FOR STUDYING NERVE GROWTH, NEUROPATHY, AND NEUROVASCULAR REMODELLING

Paul Wieringa[P][1], Afonso Malheiro[1], Abhishek Harichandan[1], Joyce Bernardi[2], Adrián Seijas Gamardo[1], Gonda F. Konings[3], Andrea Romano[3], Carlos Mota[1], Lorenzo Moroni[1]

[1] *Complex Tissue Regeneration Department, MERLN Institute, Maastricht University*

[2] *CARIM Institute, Maastricht University*

[3] *3.GROW - School for Oncology and Developmental Biology, Dept. Obstetrics and Gynaecology, Maastricht University*

Corresponding author's email: p.wieringa@maastrichtuniversity.nl

We present a scaffold-based in vitro platform to create a 3D biomimetic sensory nerve model with applicability for pathology modelling, drug testing, tissue innervation and patterning. Starting from an optimized high throughput 3D differentiation protocol, we first generate uniform and homogenous human iPSC-derived nociceptive sensory neurons and validate based on marker expression and electrophysiology. To recreate the three-dimensional (3D) anisotropy and myelination of native neural tissue, we seed primary rat schwann cells (SCs) seeded onto an electrospun scaffold and embed in a fibrin hydrogel to provide three-dimensionality. The versatility of this scaffold was shown using the PC12 cell line, rat dorsal root ganglions (DRGs), human IPSC-derived nociceptors as possible different neural cells. All neurite growth was well aligned and maintained mature myelin segments. Fibrin provided a 3D environment with increased outgrowth compared to 2D cultures, with over 6.5 mm of axonal growth densely packed within a 3D matrix. Consistent anisotropic neurite growth was observed throughout the gel, suggesting a unique indirect ‘at a distance’ guidance mechanism. Using this 3D co-culture platform to model diabetic neuropathy, the demyelinating effects of hyperglycemia were shown and therapeutic drugs were evaluated to prevent or counter demyelination. We further show the versatility and utility of this platform by demonstrating the introduction of pancreatic pseudoislets and endometrium organoids, to create target tissue innervation models. Finally, we incorporate a vascular network to investigate neurovascular (NV) interactions and show, for the first time, the impact of SCs on in vitro vascular networks formation and vessel orientation.

Abstract **#1461**

DRUG RELEASING HIERARCHICAL SCAFFOLDS FOR HUMAN EARDRUM RECONSTRUCTION

Shivesh Anand$_P$[1], Cemre Günday[2], Sara Munafò[1,3], Nazende Günday Türeli[2], Serena Danti[3], Lorenzo Moroni[1], Carlos Mota[1]

[1] *Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands*

[2] *MyBiotech GmbH, Industriestr. 1B, Überherrn, Germany*

[3] *Department of Civil and Industrial Engineering, University of Pisa, Pisa, Italy*

Corresponding author's email: s.anand@maastrichtuniversity.nl

The human eardrum or tympanic membrane (TM) is a thin, concave tissue located at the end of the ear canal [1]. It is responsible for receiving sound vibrations from the outer air and transmitting them to the middle ear. Perforated TMs are the most common injury to the human ear, resulting in a partial or complete hearing loss due to inept sound transmission [2]. In this work, we investigate the integration of tissue engineering approaches with targeted drug delivery for a complete reconstruction of the damaged eardrum.

Ciprofloxacin-encapsulated poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles were synthesized with two distinct approaches, namely double-emulsion and nanoprecipitation, by applying an automated microjet reactor technology. A co-electrospinning/electrospraying strategy was implemented to incorporate these drug-loaded nanoparticles within an electrospun mesh of poly(ethylene oxide terephthalate)/poly(butylene terephthalate) [3]. Subsequently, a three-dimensional pattern mimicking the microanatomy of the native TM was deposited over it using additive manufacturing. Homogeneous distribution and adhesion of the PLGA nanoparticles was confirmed on the fabricated scaffolds; following which, the release kinetics of ciprofloxacin was evaluated. Optimization of the loading parameters are currently under investigation to ensure a sustained drug delivery, capable of preventing all on-site infections upon transplantation. Finally, preliminary cytocompatibility studies performed with human mesenchymal stromal cells revealed no noticeable effect of the ciprofloxacin-encapsulated nanoparticles on cellular attachment and proliferation.

This work introduces a combination of hierarchical biofabrication strategy with nanoparticle drug delivery systems to conduct a full eardrum reconstruction. The nano-biofabricated TM scaffolds will be ultimately validated based on their antimicrobial and anti-inflammatory responses.

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Abstract **#1463**

INVESTIGATION OF E-CADHERIN-MEDIATED MECHANOSENSING ON DIFFERENT MICROSTRUCTURES

Abigail Dee Chen$_P$[1], Nan Huang[1], Barbara Pui Chan[1]

[1] *Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong Special Administrative Region*

Corresponding author's email: bpchan@hku.hk

Effects of mechanical stiffness in the cell niche has typically been evaluated via modulating ECM stiffness. However, cellular stiffness may also play an important role in various cellular processes, as cell junction proteins such as beta-catenin can function both as a mechanosensor and a signalling protein.

Using multiphoton fabrication, we studied the effects of modulus and 3D mechanical confinement with E-cadherin coated substrates on cell fates. Cells were seeded onto two types of structures: flat matrix structures which presented E-cadherin only, or a 3D checkerboard structure with laminin on the base and E-cadherin on the sides to observe monolayer formation and fidelity.

On the E-cadherin coated flat matrix structures, beta-catenin spreading was observed to correlate with F-actin differently in low- and high- modulus conditions. On high-modulus E-cad substrates, diffuse beta-catenin was observed and actin localization was observed at protrusions. On low-modulus E-cad substrates, cells exhibited sporadic spikes or puncta of beta-catenin that co-localized with actin spikes.

On the checkerboard structures, cells spread and were rounded, filling up the space on the structures. Compared to laminin-only controls, E-cadherin and beta-catenin staining had lower intensity. This suggests that cells may regulate cadherin expression when exposed to 3D mechanical confinement with apicobasal cues.

Combined, these results improve our understanding of mechanosensing at E-cadherin junctions, as a first step to understanding the role of cell stiffness in processes such as EMT and epithelial cell development.

Abstract **#1464**

DEVELOPMENT OF AN EX-VIVO MODEL FOR FUNCTIONAL VASCULAR TISSUE

Rolando Szilveszter Matos[1], Davide Maselli[1], John McVey[1], Paola Campagnolo$_P$[1]

[1] *University of Surrey*

Corresponding author's email: p.campagnolo@surrey.ac.uk

Introduction: Routine cardiovascular interventions such as balloon angioplasty, cardiac bypass and stenting, provoke vascular activation and remodelling often requiring further interventions(1). Current models fail to account for physical stimuli such as flow and the interaction between cells and the extracellular matrix, which are essential for vascular tissue maintenance and function(2). Methods: MultiJet Fusion 3D-printing technology was used to create our novel bioreactor insert (EasyFlow) conveniently fitted on a 50ml centrifuge tube. Porcine carotid arteries were excised and cultured at different flowrates using EasyFlow for up to 7 days. Control tissue was cultured under static conditions. Some vessels underwent injury using balloon catheter at day 0. Tissues were collected at different time points and analysed by immunofluorescence and confocal microscopy.Results: We have successfully designed and manufactured a cost-efficient and versatile perfusion system. The EasyFlow perfusion adaptor takes advantage of 50ml centrifuge tubes isolating the reaction space from the environment and reducing the culture volume. The adaptability of our design accommodates vessels of different sizes and origins while the self-contained nature of EasyFlow allows efficient parallel-cultures and minimal media consumption, ideal for pharmacological studies. Culture of porcine arteries in EasyFlow preserve the endothelial coverage and the smooth muscle organisation, as compared to static culture. Upon balloon injury, tissues cultured in flow recapitulated the hallmarks of remodelling, such as intimal denudation, smooth muscle cell disarray and activation.Conclusions: This work lays the basis for ex-vivo future investigations into how vessel wall resident cells contribute to the pathological remodelling of blood vessels.

Keywords

3D printing; Disease models; Translational research

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Abstract **#1465**

SINGLE WALL CARBON NANOTUBE REINFORCED COLLAGEN-CHITOSAN HYDROGELS: PROMISING APPROACH FOR BONE TISSUE REGENERATION

Kulwinder Kaur[P][1], Ciara Murphy[1]

[1] *Department of Anatomy, Tissue Engineering Research Group, Royal College of Surgeons in Ireland, 123 St. Stephens Green, Dublin 2, Ireland*

Corresponding author's email: KulwinderKaur@rcsi.com

The development of highly biomimetic scaffolds in terms of composition and mechanical structure is particularly relevant for bone tissue engineering. Carboxylated single walled carbon nanotubes (SWCNT) are attractive for use due to their high aspect ratio combined with outstanding mechanical properties. This project investigates the use of mechanically robust SWCNT to reinforce collagen-chitosan hydrogels, to develop thermoresponsive hydrogels with appropriate mechanical properties and bioactivity, for use in load-bearing bone applications. Hydrogels comprising of a collagen-chitosan polymeric matrix, incorporating SWCNT, were prepared by mixing solubilized type I collagen and medium molecular weight chitosan with solutions of SWCNT at concentrations of 0, 0.5, 1 and 2 wt% in presence of β-glycerophosphate. The effect of SWCNT composition on the chemical, mechanical and thermoresponsive properties of the hydrogels was investigated using different physiochemical techniques including UV-visible spectroscopy, rheology, FTIR, Raman Spectroscopy, SEM and TEM analysis. It was determined that increasing concentration of SWCNT led to increased mechanical strength. Furthermore, the strong molecular interactions formed between polymeric matrix and SWCNT led to increased porous interconnectivity. The degradation ratio was varied by changing the concentrations of Collagen/ Chitosan and SWCNT. Thermoresponsive properties were achieved in all hydrogel groups by adjusting pH~7.4 with β-glycerophosphate, gelation occurs at ~36-37 degree Celsius within 5 minutes. Ongoing work is investigating the bioactivity of the hydrogels in terms of human mesenchymal stem cell viability, proliferation and osteogenic differentiation. These results demonstrate the potential of the hydrogels for minimally invasive applications in the field of bone tissue engineering and regeneration.

Keywords

Osteoporotic vertebral fractures ; Injectable hydrogels ; mechanically robust

Abstract **#1466**

EVALUATION OF BIOMAGNETIC HUMAN TISSUE SUBSTITUTES FOR TISSUE ENGINEERING APPLICATIONS. AN IN VIVO STUDY

Fernando Campos$_P$[1], ISMAEL A. RODRÍGUEZ[2], MODESTO T. LÓPEZ-LÓPEZ[3], CARMEN GONZÁLEZ-GALLARDO[4], RICARDO FERNÁNDEZ-VALADÉS[5], M. CARMEN SÁNCHEZ-QUEVEDO[1], INGRID GARZÓN[1], MIGUEL ALAMINOS[1], ANTONIO CAMPOS[1], VÍCTOR CARRIEL[1]

[1] Tissue Engineering Group, Department of Histology, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain

[2] UNIVERSIDAD NACIONAL DE CÓRDOBA, ARGENTINA

[3] Department of Applied Physics, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain

[4] Division of Ophthalmology, Univ. Hospital San Cecilio, Granada, Spain

[5] Division of Pediatric Surgery, Univ. Hospital Virgen de las Nieves, Granada, Spain

Corresponding author's email: malaminos@ugr.es

BACKGROUND: Biomaterials can be functionalized using nanomagnetic particles (NMP) able to increase the biomechanical and functional properties of bioartificial tissues (1). However, the in vivo biodistribution of NMP used in tissue engineering are not fully understood.

METHODS: Fibrin-agarose tissue-like substitutes containing magnetoferric NMP with a diameter of 70-80 nm were generated and subcutaneously grafted in Wistar rats. Biodistribution was assessed by magnetic resonance imaging (MRI) and histology, and major biochemical parameters were quantified in peripheral blood.

RESULTS: MRI and histological analyses showed that NMP stayed at the grafting site after 3 months and did not migrate to distal organs. In addition, a biochemical analysis of relevant parameters in blood revealed that all hepatic and renal function parameters were within normal values and were not altered by the bioartificial tissue implant.

CONCLUSIONS: These results suggest that the biomagnetic bioartificial tissues used in this work were safe for the animals and tended to stay at the implant site, while providing novel functions and properties to the fibrin-agarose tissue-like grafts.

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Abstract **#1469**

COMPRESSION LOADING INDUCED FORMATION AND MATURATION OF 3D-MATRIX ADHESIONS (3DMAS) IN HUMAN MESENCHYMAL STEM CELLS

Barbara Mechanical Chan[P][1], CW Li[1], YT Lau[1], KL Lam[1]

[1] *Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong*

Corresponding author's email: bpchan@hku.hk

Cells are known to sense and respond to mechanical signals including substrate compliance and mechanical loading. Cell-matrix adhesions are important molecular mechanosensors at the interface between cells and the matrix microenvironment. However, how external mechanical force affects the cell-matrix interactions particularly with the formation and maturation of cell-matrix adhesions is largely unknown.

Here, using a previously developed mechanical loading device [1], human mesenchymal stem cells (hMSCs)-collagen microtissues [2] were subjected to compression loading for both short term and long term, while the formation and maturation of integrin-based cell matrix adhesions were investigated.

Specifically, we found that integrin αV binding, focal a¬¬dhesion formation, and subsequent FAK activation were upregulated upon short-term dynamic compression. This compression-induced FAK signaling also leads to YAP activation. While long-term compression induced the formation and maturation of α5-integrin based adhesions, which are characterized as elongated 3D-matrix adhesions (3DMAs) with composition and morphology different from that of the 2D focal adhesions. Most importantly, compression loading-based pre-conditioning of hMSCs in the 3D micro-tissues induced the formation of mature integrin α5-dependent 3DMAs and potentiated the osteogenic differentiation of hMSCs. Collectively, this work demonstrated that active mechanical stimulation (compression) alters cell-matrix interactions at the cell-material interfaces in a dynamic manner and affects cell fate determination, demonstrating the significance of loading-based functional tissue engineering.

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Abstract **#1470**

THE EARLY EFFECTS OF SCAFFOLD-FREE CONSTRUCTS OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS ON TENDON-BONE HEALING AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION IN A RABBIT MODEL

Kotaro Higa[P][1], Hidetoshi Matsuda[1], Fuminari Uehara[1], Takashi Toma[1], Chinatsu Azuma[1], Daiki Murata[2], Koichi Nakayama[2], Kotaro Nishida[1]

[1] *Department of Orthopedic Surgery, Graduate School of Medicine, University of the Ryukyus*

[2] *Department of Regenerative Medicine and Biomedical Engineering, Faculty of Medicine, Saga University*

Corresponding author's email: kotarohiga@yahoo.co.jp

Introduction: Adipose tissue-derived mesenchymal stem cells (AT-MSCs) have received attention as a tool to enhance graft healing in ACL reconstruction. Nakayama developed a novel method to create scaffold- free tubular tissue from multicellular spheroids using a “Bio-3D printer”-based system. This system enables the creation of pre-designed three-dimensional structures using a computer robotics system. With this system, we created a tubular AT-MSCs constructs and studied its effect on tendon-bone healing in a rabbit ACL reconstruction model.

Methods: 12 white rabbits were used in this study. Interscapular fat tissue was harvested and tubular AT-MSCs constructs were made using bio-3D printer. ACL reconstruction with semitendinosus tendon autograft was performed. In the right knee, AT-MSCs constructs were implanted into the femoral bone tunnel (implantation group). In the left knee, only ACL reconstruction was performed (control group). Each 6 rabbits were sacrificed at 3 and 6 weeks postoperatively. 5 of the 6 rabbits were used for biomechanical and μCT analysis, remaining 1 were used for histological observation.

Results: On biomechanical analysis, the ultimate failure load of implant group at 3 and 6 weeks were significantly greater than those of control group. On μCT analysis, the volume of bone formation in the femoral bone tunnel in implant group was significantly smaller than control group at 3 weeks, but increased significantly at 6 weeks and showed no significant difference with control group.

Conclusion: Implantation of AT-MSCs constructs into femoral bone tunnel enhanced early pull-out strength of tendon graft, and promoted osteogenesis after 3 weeks.

Keywords

Anterior cruciate ligament reconstruction; Bio-3D printers; Adipose tissue-derived mesenchymal stem cells

Abstract **#1471**

MUSCADINE GRAPE EXTRACT IMPROVES SKELETAL MUSCLE REGENERATION THROUGH MACROPHAGE ATTENUATION

James M Poteracki$_P$[1], Yu Zhou[1], Patricia Gallagher[2], Ann Tallant[2], Tracy Criswell[1]

[1] *Wake Forest Institute of Regenerative Medicine*

[2] *Wake Forest University Dept. of Hypertension*

Corresponding author's email: jpoterac@wakehealth.edu

Introduction

Compartment Syndrome (CS) is a severe skeletal muscle injury requiring surgical intervention (fasciotomy). CS occurs after a primary injury to a muscle, resulting in increased intra-compartmental pressure (ICP), blood vessel occlusion, and tissue ischemia. Fasciotomy decreases ICP, but results in functional deficits and tissue fibrosis. Muscadine grape extract (MGE) is a mixed polyphenol compound with anti-inflammatory properties which may attenuate the macrophage response after injury and activate tissue regeneration.

Methods

In vivo: Using a rat model of CS with fasciotomy, MGE was administered in the drinking water 2 weeks prior to CS injury and throughout the experiment. Tibialis anterior (TA) function was measured pre- and post-injury and tissue collected for histological evaluation. In vitro: RAW 264.7 macrophages were cultured with MGE to determine if MGE could attenuate the inflammatory cytokine response, specifically through NF-κB inhibition. C2C12 myoblasts and primary muscle progenitor cells (MPCs) were cultured with MGE to determine if cell proliferation and myotube fusion were altered by MGE.

Results

Increased maximal force was detected in MGE treated rats pre and post CS injury. At 3 days post-injury, the number of Pax7+ satellite cells and CD68+ macrophages were decreased, while MyoG+ myofibers were increased compared to untreated controls. In vitro MGE treated macrophages decreased IL-6 mRNA expression compared to untreated controls.

Conclusion

These data suggest that MGE altered the macrophage phenotype and satellite cell response in vivo which may be due to altered cytokine production, as observed in vitro. MGE may be a novel therapeutic for skeletal muscle regeneration.

Keywords

Immunomodulation; Polyphenol; Rat Model

Abstract **#1472**

REMODEL – EXPANDING NON-ANIMAL RESEARCH USING ADVANCED IN VITRO MODELS AND ORGANOIDS

Marta Alves da Silva$_P$[1], Cristina Barrias[1], Sina Bartfeld[2], Roos Masereeuw[3], Celso Reis[1], Margarida Saraiva[1], Hans Wandall[4], Anna Olsson[1]

[1] *I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

[2] *University of Wuerzburg, Wuerzburg, Germany*

[3] *Utrecht University, Utrecht, Netherlands*

[4] *University of Copenhagen, Copenhagen, Denmark*

Corresponding author's email: martas@i3s.up.pt

REMODEL is an H2020 project dedicated to building capacity to work with organoids and advanced 3D models in biomedical research. Biomedical research faces a need for replacement of animal experiments by reliable, safe and accurate in vitro models that better recapitulate the structure and function of human tissues than present 2D models. Among emerging in vitro models, organoids are especially interesting as they present the 3D organization that is characteristic for a certain tissue in vivo(1). Organoids bridge 2D cultures and in vivo models, providing a near-physiological model system for studying cells and tissues in a variety of contexts, e.g. patient-derived organoids for studying human diseases that are difficult to model in animals(2). While there is an enormous interest among scientists to access these model systems, the laboratory skills required to successfully develop organoids for different research purposes requires training. Existing training in model systems is focused either on animal use in research or non-animal models for toxicology testing. REMODEL aims to overcome the gap by developing advanced training in organoid and organoid-like systems for students and early researchers. Starting in 2020, we are organizing workshops and developing tools for blended learning to build a strong European network on 3D in vitro models for cancer, infection and tissue regeneration. Through research collaborations we also intend to tackle technical challenges such as the absence of an immune system and a vasculature within organoid cultures. Herein, we will present training models and outcomes based on the experience of the project first year.

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Abstract **#1473**

IN VIVO BONE REGENERATION USING A LAYER-BY-LAYER ASSEMBLY OF PLGA-HA MEMBRANE LOADED WITH SVF FROM ADIPOSE TISSUE

Joanna Babilotte[P][1], Benoit Martin[1], Olivier Chassande[1], Sylvie Rey[1], Martine Saint-Marc[1], Damien Le Nihouannen[1], Sylvain Catros[2]

[1] *Univ. Bordeaux, INSERM, BioTis U1026, 33076 Bordeaux, France*

[2] *Univ. Bordeaux, INSERM, BioTis U1026, CHU Bordeaux, Dentistry and Oral Health Department, 33076 Bordeaux, France*

Corresponding author's email: joanna.babilotte@gmail.com

Current bone tissue engineering (BTE) strategies are based on porous biocompatible scaffolds seeded with tissue-specific cells. The conventional approach involves seeding cells onto a macroporous scaffold and expects cell colonization to form composite tissue constructs. Many limitations have been observed using this approach, due to slow vascularization, limited diffusion of nutrients, low cell density and non-uniform cell distribution. Previous studies have shown that the Layer-by-Layer (LBL) fabrication approach, based on the assembly of small-seeded blocks, provided more efficient cell repartition in 3D compared to conventional methods [1,2].

The aim of this work was to evaluate the in vivo bone formation using cellularized macroporous scaffolds or LBL assembly of cellularized PLGA-HA membranes. We have developed a new material, made of medical-grade Poly(lactic-co-glycolic) acid (PLGA) mixed with 10% (w/w) hydroxyapatite nanoparticles (HA) for 3D printing by Fused Deposition Modelling (FDM) [3]. Materials were seeded with rat stromal vascular fraction isolated from adipose tissue. Both approaches demonstrated favorable tissue formation and relevant cellular responses for BTE applications. The results indicated that the LBL approach could be suitable for BTE, in order to promote homogenous cell distribution inside the scaffold.

Keywords

Polymer; Hydroxyapatite; Layer-by-Layer

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Abstract **#1474**

A NOVEL SCAFFOLD WITH HIGH GLYCOSAMINOGLYCAN/HYDROXYPROLINE RATIO FOR INTERVERTEBRAL DISC REGENERATION

Xingxing YANGP[1], Barbara Pui Chan[1]

[1] *Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong Special Administrative Region, China*

Corresponding author's email: bpchan@hku.hk

INTRODUCTION: In degenerated disc, nucleus pulposus (NP) lose its ability to maintain the cell function, followed by loss of glycosaminoglycan (GAG) content and reduction in disc height. It has been reported that the GAG/Hydroxyproline ratio in health young adult NP is 27:1 and decreased to 5:1 in aged [1]. Methods to enhance GAG incorporation has been intensively studied, but the GAG/HYP ratio of modified collagen-GAG scaffolds could not exceed to 4.5:1[2-3], which is below that of the NP of a young adult. Hence, development of scaffold with high GAG incorporation that mimic the native NP would be a useful strategy for disc regeneration. In this study, we report a novel scaffold, aminated collagen-aminated hyaluronic acid-GAG (aCol-aHA-GAG), with comparable GAG/HYP ratio and similar ultrastructure to native NP, good biocompatibility and cell phenotype maintenance ability.

EXPERIMENTAL METHODS: The aCol-aHA-GAG scaffold with different composition were fabricated before evaluating the GAG/HYP ratio, GAG release, ultrastructure, mechanical properties of the resultant coprecipitate structures. Bovine nucleus pulposus cells (bNPCs) were encapsulated into the aCol-aHA-GAG scaffold and cell viability, extracellular matrix deposition, cell phenotype maintenance as well as gene expression of phenotype marker was studied in detail.

RESULTS AND DISCUSSION: The GAG/HYP ratio of the scaffold was 20:1, approaching that of young adult native NP. SEM image showed bead-like and TEM image showed brush-like structure. Safranin-O staining of the bNPCs encapsulated scaffold showed extensive GAG distribution.

CONCLUSIONS: This study demonstrates that aCol-aHA-GAG structurally and functionally bio-mimic the native NP, would be an ideal scaffold for disc regeneration.

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Abstract **#1475**

GUIDING MACROPHAGE PHENOTYPE BY RATIONALLY-DESIGNED SCAFFOLDS FABRICATED BY MELT ELECTROWRITING TO MODULATE INFLAMMATION

Silvia Lopa[1], Carlotta Mondadori[1,2], Amit Chandrakar[3], Paul Wieringa[3], Alberto Redaelli[2], Matteo Moretti[1,4], Lorenzo Moroni[3]

[1] *IRCCS Istituto Ortopedico Galeazzi, Cell and Tissue Engineering Laboratory, Milan, Italy*

[2] *Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milano, Italy*

[3] *Maastricht University, Department of Complex Tissue Regeneration, Maastricht, The Netherlands*

[4] *Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Lugano, Switzerland*

Corresponding author's email: silvia.lopa@grupposandonato.it

Introduction

Macrophages are immune cells able to polarize toward a pro- (M1) or anti-inflammatory (M2) phenotype, playing a key role in inflammation. The macrophage phenotype has been shown to change in response to biophysical cues. Here, we exploited melt electrowriting (MEW) to fabricate scaffolds with different 3D geometries to investigate the ability of scaffold architecture in modulating macrophage phenotype.

Methods

Polycaprolactone (PCL) scaffolds with different angles (90°, 60°, 120°) formed between adjacent fibers were fabricated by Melt Electrowriting (MEW). Unpolarized (M0) and polarized (M1, M2a, M2c) macrophages were cultured for 3 days on scaffolds (control groups: TCP and PCL films). To evaluate macrophage polarization, the release of pro- (TNF-α, IL-6, IL-1β, CXCL9, CCL5) and anti-inflammatory (IL-1RA, IL-10, IL-13, CCL22, CCL24) proteins was assessed.

Results

We selected three different pore geometries (square-, triangle-, and rhombus- grid) to obtain scaffolds with 90°, 60°, and 120° angles, respectively. The rhomboidal grid was more effective than the other configurations in promoting the release of all the analyzed anti-inflammatory factors, especially IL-10, by M0 and M2a macrophages and it was the only scaffold that significantly enhanced IL-10 secretion by M2a macrophages compared to PCL films.

Discussion & Conclusions

The rhomboidal architecture increased the secretion of anti-inflammatory factors, especially compared to PCL films without geometric cues, suggesting that rational scaffold design could be exploited to modulate the macrophage phenotype with the final aim of improving tissue healing.

Acknowledgments

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Abstract **#1476**

DEXTRAN-BASED SCAFFOLDS FOR IN-SITU HYDROGELATION: USE FOR NEXT GENERATION OF CHEMICALLY DEFINED BIOARTIFICIAL CARDIAC TISSUES

Monika Szepes[P][1], Samhita Banerjee[2], Nick Dibbert[2], Julio-Cesar Rios-Camacho[1], Andreas Kirschning[2], Ina Gruh[1], Gerald Dräger[2]

[1] *Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School*

[2] *Institut for Organic Chemistry, Leibniz University Hannover*

Corresponding author's email: szepes.monika@mh-hannover.de

Grafts produced by combining induced pluripotent stem cell (iPSC)-derivatives and tissue engineering (TE) are a promising therapeutic option for the replacement of cardiomyocytes (CM) lost due to myocardial infarction. Recent protocols allow for differentiating CMs in large scale and chemically defined (CD) manner [1]. However, most extracellular matrices used for TE in vitro are not suitable for transplantation due to immunogenicity, insufficient mechanical characteristics, or unreliable reproducibility. In search for CD substitutes of commonly used xenogeneic matrices, we disclose new dextran (Dex)-derived hydrogels with varying molecular weights suited for bioartificial cardiac tissue (BCT) development.

Dextran hydrogels were generated by an in situ gelation protocol [2] based on crosslinking via hydrazone formation. By varying the degrees of derivatization and the composition, material properties such as water storage capacity and mechanical strength could be flexibly adjusted. To modulate bioactivity, the cyclic pentapeptide RGD was coupled to the dextran backbone. A hydrogel blend with dextran and human collagen I (hColI) was added to iPSC-CMs and fibroblasts to generate BCTs.

Our results show that unlike collagen alone, Dex+hColI blends with or without RGD supported tissue formation and long term cultivation. The gel compaction, contractile strength and beating frequency of the formed BCTs were comparable to published TE constructs and transcriptome analysis revealed functional maturation of the CMs within the constructs. While, the presence of RGD did not show a beneficial improvement, we could demonstrate the feasibility of covalently crosslinked dextran hydrogels as matrix system in the field of cardiac regenerative medicine.

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Abstract **#1477**

TUNING MECHANICAL PROPERTIES BY RATIONAL DESIGN OF 3D PRINTED SCAFFOLDS FOR HEART VALVE TISSUE ENGINEERING

Klaus Kreuels[P][1], Zhaoyang Zhong[2], Petra Mela[2], Nadine Nottrodt[3], Arnold Gillner[1,3]

[1] *Chair for Laser Technology LLT, RWTH Aachen University*

[2] *Chair of Medical Materials and Implants MMI, Technical University of Munich*

[3] *Fraunhofer Institute for Laser Technology ILT*

Corresponding author's email: klaus.kreuels@llt.rwth-aachen.de

More than 42 million people worldwide suffering from heart valve disease could benefit from tissue engineered heart valves able to overcome the disadvantages of the available heart valve prostheses.[1] Utilizing highly resolved stereolithography we address the production of a scaffold for an advanced biohybrid heart valve implant with anisotropic mechanical properties mimicking those of the aortic valve. Specifically, we aim at replicating the properties of the three layers (i.e. ventricularis, spongiosa, fibrosa)[2] of the native heart valve tissue.

Polymeric scaffold structures were 3D printed using either a Caligma200UV from Cubicure employing a laser source with a wavelength λ=375nm with an acrylic photo resin or a custom-made stereolithography setup with a Nd:YAG laser source at λ=266nm and a self-developed thiol-ene-based photo resin[3]. Diverse unit cell designs were tested. Thoroughly cleaned scaffolds were mechanically characterized and tested for cytotoxicity. Finite element analysis was performed to optimize the scaffold architecture.

Mechanical properties of scaffold structures were investigated in respect of unit cell design, wall thickness and porosity, starting from a cubic lattice structure and moving to rhombohedral and Schwarz-P structures. The mechanical characterization reflected the material properties of the used polymers and of the 3D-architecture. From the aspect of material properties, polymer scaffolds from acrylic resin showed a higher Young's modulus but a lower ultimate strain than thiol-ene-based scaffolds. Furthermore, the ultimate mechanical properties of the scaffold depend on the unit cells used to construct the 3D-architecture. First cytotoxicity assessment on thiol-ene materials demonstrated that scaffolds can be used in combination with cells.

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Abstract **#1478**

HUMAN PROTEIN BASED PLATFORMS FOR 3D CELL CULTURE - A NEW ERA FOR PERSONALIZED ENGINEERING TISSUES

Catarina Custódio$_P$[1], Sara Santos[1], Inês Deus[1], Elisa Martins[1], João Mano[1]

[1] *CICECO- Aveiro Institute of Materials, Universidade de Aveiro*

Corresponding author's email: catarinacustodio@ua.pt

A physiologically relevant cell culture model is one that mimics the in vivo tissue organization and is physiologically relevant and/or patient-derived and meet the requirements of the pharmaceutical industry. We propose human-based biomaterials prepared from placenta and platelet rich plasma (PRP) to engineer humanized platforms for cell culture.(1,2,3) Human placenta based biomaterials result in non-immunogenic platforms with anti-inflammatory and pro-angiogenic properties. PRP is rich in growth factors and involved in tissue healing and angiogenesis that was herein explored to prepare bioactive hydrogels for cell culture.

Placenta was decellularized, solubilized, lyophilized, and milled. Platelet lysates (PLs) were prepared by exposing PRP to freeze-thaw cycles. The placenta extracellular matrix (hPECM)-proteins and PLs were chemically modified using a methacrylation protocol well established in our group. (1) Degree and local of modification were evaluated by mass spectrometry. The methacrylated proteins were processed in the form of a hydrogel upon irradiation with UV light.

The mechanical properties of the resulting gels can be easily tailored by varying the degree of modification and/or the concentration of the solution. The gels have proven to support distinct human derived cell cultures (stem cells, endothelial cells and cardiomyocytes), furthermore, the cells acquired an in vivo-like cell polarity and were able to invade the matrix, forming complex interconnected cellular networks.

We propose human based materials, as innovative, versatile platforms for cell culture, microtissue development and disease modeling. It offers the possibility to be personalised (using the patient's own proteins) according to the end-user needs with high potential for clinical translation.

Keywords

human protein biomaterals; 3d cell culture; personalized tissue engineering

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Abstract **#1479**

NEW PHOTOCLEAVABLE HYDROGELS FOR HIGH-RESOLUTION SUBTRACTIVE BIOFABRICATION

Wanwan Qiu[P][1], Ralph Müller[1], Xiao-Hua Qin[1]

[1] *ETH Zurich, Institute for Biomechanics*

Corresponding author's email: wanwan.qiu@hest.ethz.ch

Phototunable hydrogels are important materials for tissue engineering and regenerative Medicine [1]. Recent advances have enabled researchers to grow functional tissues in 3D hydrogels with fine-tuned biochemical and biophysical properties. The integration of these materials with additive manufacturing techniques such as two-photon polymerization (2PP) allows 3D construction of complex structures. However, low throughput of 2PP remains a major challenge for scaled biofabrication at high spatial resolution.

To address these limitations, we seek to combine photocleavable hydrogels with subtractive two-photon photolithography to fabricate biomimetic microstructures during 3D culture. So far, the o-nitrobenzyl ether [2] and coumarin derivatives [3] are often used to prepare photocleavable hydrogels. However, these compounds suffer from rather limited efficiency for two-photon absorption. As a result, very high laser dosage and long irradiation times are required for photoscission parameters at which living cells are prone to be damaged [4].

In this contribution, we will present the design and synthesis of novel photocleavable hydrogels and their applications for photodynamic 3D culture. To improve the efficiency of these materials, we employ new concepts in photochemistry by introducing chemical groups with large two-photon absorption cross sections such as the long-conjugated oxime-ester derivatives [5] to cell-compatible polymers such as polyethylene glycol and hyaluronan. The mechanical properties of these gels are studied by in situ photo-rheology, while two-photon hydrogel degradation is investigated on a confocal laser scanning microscope. Preliminary results of subtractive high-resolution biofabrication will be presented in a 3D bone cell culture. The promise and challenge of this platform will be discussed.

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Abstract **#1481**

BIOINSPIRED RGD-ENGINEERED BACTERIOPHAGE NANOFIBERS PROVIDE A VASCULAR AND AN ANTIOXIDANT NICHE

Kshitiz Raj Shrestha[P][1,2,3], Su-Nam Jeong[1,2], Seon Yeong Chae[1,4], So Young Yoo[1,2]

[1] *BIO-IT Foundry Technology Institute, Pusan National University, Busan, Republic of Korea*

[2] *Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, Republic of Korea;*

[3] *Department of Biotechnology, National College, Tribhuvan University, Kathmandu, Nepal (Current address)*

[4] *Department of Nano Fusion Technology, Pusan National University, Busan, Republic of Korea*

Corresponding author's email: kshitizrajshrestha@gmail.com

Instructive tissue engineering biomaterials provide a vascular niche and protect oxidative stress in injured tissue. In this study, we exploited bioinspired bacteriophage nanofibers, previously recognized by their biochemical and structural cues inducing angiogenesis, as an antioxidant tissue engineering material. We demonstrated that topological cues of Arg-Gly-Asp (RGD)-engineered bacteriophage nanofibers provide angiogenic niches and cytoprotective functions against cellular oxidative stress with increased expression of antioxidant enzymes heme oxygenase-1 (HO-1) and NAD(P)H-quinone oxidoreductase 1 (NQO1) via the extracellular-signal-regulated kinase (ERK)−nuclear factor erythroid 2−related factor2 (Nrf2)-mediated signaling pathway, where a high density of RGD cues on the phage body support efficient interaction of cells with phage cues.

Abstract **#1482**

PROCESS CONTROL OF THE MATURATION OF BIOHYBRID HEART VALVES

Maximilian Werner[P][1], Kirsten Voß[2], Vytautas Kucikas[3], Dirk Abel[2], Marc A.M.J van Zandvoort[3], Stefan Jockenhövel[1], Thomas Schmitz-Rode[1]

[1] *Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany*

[2] *Institute of Automatic Control, RWTH Aachen University, Germany*

[3] *Institute for Molecular Cardiovascular Research (IMCAR), Interdisciplinary Centre for Clinical Research, RWTH Aachen University, Aachen, Germany*

Corresponding author's email: werner@ame.rwth-aachen.de

In cardiovascular tissue-engineering applications, many tissues and organs require long maturation times in bioreactors, where different physical, biochemical and cell-source related effects influence the proliferation of cells and extracellular matrix formation of the construct. In case of heart valves, the conditioning phase includes a mechanical stimulation through an increasing pulsatile flow that needs different sensors and actuators for adjustment and monitoring. The aim of this study was to develop a centrally organized control unit for the experimental equipment that autonomously controls the conditioning process without the need of manual intervention.

Therefore, the software-controlled maturation process was elaborated using the example of an aortic heart valve. The experimental setup includes a feedback-controlled software that is fed by sensor data including pressure transducers, a backflow measurement unit, a highspeed-camera and an ultrasound probe as well as a multi-photon endoscope. Pulsatile movement of the heart valve was provided by actuators controlling medium flow and the compliance volume.

The conditioned heart valves reveal a more homogeneous tissue and, among the experimental batches, less variation of the tissue development. The majority of previously manual steps were implementable in an autonomous-working process control that reduced the required working times. Besides, the current maturation state of the heart valve was transparently traceable at every point through the logged sensor data. In summary, the study showed that the dynamic conditioning of tissue-engineered heart valves is automatable. The experimental setup may be also suitable for other bioreactor applications in tissue engineering.

Abstract **#1483**

NANO-TECHNOLOGICAL APPROACH FOR TAGGING MSC-DERIVED EVS FOR CLINICAL THERAPY

Maria Elisabetta Federica Palamà[P][1], Marta Nardini[2], Arantxa Albornoz[3], De Miguel Ignacio[3], Romain Quidant[3], Katia Cortese[1], Maria Cristina Gagliani[1], Martin Leahy[4], Maddalena Mastrogiacomo[2], Chiara Gentili[1]

[1] *Department of Experimental Medicine (DIMES), University of Genoa, Genoa, Italy*

[2] *Department of Internal Medicine and Medical Specialties (DIMI), University of Genoa, Genoa, Italy*

[3] *Institute of Photonic Sciences (ICFO), Barcelona, Spain*

[4] *National University of Ireland Galway (NUIG), Galway, Ireland*

Corresponding author's email: elisabettapalama@gmail.com

Osteoarthritis (OA) is a common chronic joint disease and cause of disability worldwide. MSCs-derived extracellular vesicles (EVs) showed ability in modulation and treatment of OA. Nevertheless, a key obstacle to clinical acceptance of EV-based therapies is the uncertainty surrounding their distribution after transplant.

In this scenario, we developed a novel tool for tagging EVs, using nanostars (NS). NS are gold nanoparticles with branching arms (like a star), thanks to which they can be detected by optoacoustic imaging (OAI). OAI utilises the optoacoustic effect: nanosecond pulses of laser light illuminate the tissue; the resulting pressure wave is detected using ultrasound sensors and converted into an image. NS were carboxylated (NS-COOH), to enhance their binding ability, and chitosan-coated (NS-COOH-CHS) to avoid their clustering.

We isolated human MSCs and cultured them in a heparin-free human platelet lysate (PL), allowing their future use for clinical intent. We isolated EVs and characterized for size, morphology and content by non-conventional flow cytometry, TEM and western blot. NS-COOH and NS-COOH-CHS were added at 400pM and 800pM to EVs for different exposure time. The labelling was investigated by TEM to optimize the tagging protocol. In vitro analyses were performed to assess the functionality of MSC-EVs and NS-tagged MSC-EVs in an in vitro OA model. MSCs derived EVs are indeed able to revert the IL-1α-induced inflammation in human articular chondrocytes.

All these strategies could finally represent a novel tool for regenerative medicine, allowing to follow cells and EVs in vivo in small and large animal models.

Keywords

Extracellular vesicles; Nanoparticles; Platelet lysate

Abstract **#1484**

GENERATION OF MATURE HIPSC DERIVED HEPATOCYTES WITH HUMAN INTESTINAL MICROBIOME: A NATURE-INSPIRED PROTOCOL

Joana I. Almeida[1,2,3], Pedro Vicente[1,2], Paula M Alves[1,2], Pedro M. Baptista[3,4,5,6,7], Margarida Serra[1,2]

[1] *IBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*

[2] *ITQB NOVA - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

[3] *IIS Aragón - Instituto de Investigación Sanitaria de Aragón, Zaragoza, Spain*

[4] *CIBERehd - Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas, Madrid, Spain*

[5] *Fundación ARAID, Zaragoza, Spain*

[6] *Instituto de Investigación Sanitaria de la Fundación Jiménez Dias, Madrid, Spain*

[7] *Department of Biomedical and Aerospace Engineering, Universidad Carlos III de Madrid, Spain*

Corresponding author's email: joana.almeida@ibet.pt

Hepatocyte-like cells derived from human induced pluripotent stem cells (hiPSC-HLC) hold great promise as a new cell source of hepatocytes for preclinical drug development and regenerative medicine (1). Nonetheless, the generated hiPSC-HLC are still immature when compared to their adult counterparts. Recapitulating liver maturation in vitro is complex, as this physiological process takes approximately 2 years after birth and involves a wide range of biological events. The human gut microbiome, which is thought to be established early in the fetal stage, has been strongly associated with the acquisition of hepatic functionality (2). Herein, we developed a nature-inspired protocol to generate more mature hiPSC-HLC through the use of human microbiome.

hiPSC were differentiated into HLC using a 3D culture strategy in stirred-tank bioreactors (3). After differentiation, hiPSC-HLC were treated for 6 additional days with intestinal microbiota secretome generated from neonatal or adult samples. Our results showed that HLC generated in 3D culture exhibited higher expression of some hepatic hallmarks (CYP3A4, A1AT, HNF4a, ALB), an increase in the albumin production, higher uptake and release of indocyanine green when compared with the HLC generated in 2D monolayers. Treatment with microbiome secretome induced upregulation of CYP3A4, CYP2C9, HNF4a and ALB in HLC, which was not verified in cells cultured in medium only or supplemented with secretome from a donor under antibiotic treatment. Interestingly, the secretome from neonatal origin showed to be more efficient in driving HLC maturation than the adult formulation.

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Abstract **#1485**

HUMAN ARTICULAR CHONDROCYTE RE-DIFFERENTIATION IN 3D-PRINTED PLATELET RICH PLASMA (PRP) AND ALGINATE BASED BIOINK

Maddalena Ghelardoni[P][1], Simonetta Carluccio[1], Maria Elisabetta Federica Palamà[1], Chiara Gentili[1]

[1] *Department of Experimental Medicine (DIMES), University of Genoa, Italy*

Corresponding author's email: madda.ghela@gmail.com

Tissue engineering strategies for clinical treatments of cartilage defects are mainly based on transplantation of in vitro expanded autologous chondrocytes in the patient damaged tissue. However, their dedifferentiation during in vitro expansion often leads to suboptimal therapeutic outcomes [1]. Three dimensional (3D) culture systems can revert this dedifferentiated state and re establish chondrogenic phenotype [2]. In this scenario, 3D-bioprinting technology allows to create a patient-shaped grafts starting from cell-laden bioink, obtaining a more physiological environment for transplanted cells, promoting tissue regeneration. Thus, human articular chondrocytes (hACs), isolated from patient tissue biopsies and expanded in vitro in monolayer, were 3D-bioprinted in alginate or alginate Platelet Rich Plasma (PRP)-mixed bioinks. In vitro cell morphology, viability, growth and chondrogenic differentiation were investigated. 3D culture in both cases significantly increased the expression of chondrogenic markers compared to 2D condition. Furthermore, the addition of PRP to alginate up-regulated the expression of these markers. PRP functionalization increased hACs viability and growth compared to alginate alone. 3D constructs were implanted in nude mice to analyze the chondrogenic differentiation in vivo, showing a slight cartilage tissue-like organization after two months. Finally, 3D-bioprinting of hACs allowed them to recover chondrogenic phenotype and the embedding in PRP supplemented bioink supported chondrogenic culture inside the printed constructs. In view of future clinic translation, the choice of cell source for 3D-bioprinting of patient-specific grafts will have to be oriented towards cell types with higher potential, such as chondro progenitors, than mature hACs in order to ensure a successful cartilage regeneration and repair.

Keywords

3D-bioprinting; articular chondrocytes; Platelet Rich Plasma

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Abstract **#1487**

IMAGE-BASED TWO-PHOTON BIOFABRICATION OF BONE CELL MODELS

Xiao-Hua Qin$_P$[1], Christian Gehre[1], Ximena Sutter[1], Philipp Campos[1], Ralph Müller[1]

[1] *ETH Zurich, Institute for Biomechanics*

Corresponding author's email: qinx@ethz.ch

Developing miniature bone cell models is key to tissue engineering and disease modelling. Bone has a sophisticated internal architecture across several length scales. Recapitulating the structural complexity in bone requires the development of high-resolution biofabrication techniques that faithfully recreate tissue architecture down to the micrometer-scale accuracy. One promising approach is to combine computer models derived from biomedical imaging data with light-based additive manufacturing (AM) techniques. Using light as the stimulus, photopolymer solutions can be locally solidified through lithographic voxel-by-voxel patterning in accordance with computer models. However, existing high-resolution AM techniques by means of two-photon polymerization often suffer from lengthy fabrication process and poor cell-compatibility.

Here, we present an image-based subtractive manufacturing process for creating cell guidance cues in biocompatible hydrogels. To this end, new computer models that mimic the topology of lacuno-canalicular network (LCN) in bone are developed by sequential immunostaining and confocal microscopic imaging of osteocytes in mouse bone specimen. These models are processed into stereolithography files through image processing with MATLAB. Using two-photon subtractive 3D microprinting, we demonstrate the fabrication of LCN-mimicking microstructures inside a photodegradable polyethylene glycol hydrogel at high spatial resolution. The structure fidelity is highly dependent on the laser processing parameters such as laser power and writing speed, whereas permeability assays with fluorescently labelled proteins shows that the printed microstructures are accessible to fluid transport. Lastly, preliminary data on subtractive 3D microprinting in the presence of living bone cells and guided 3D cell growth will be presented.

Abstract **#1488**

CELLS AND MICROPARTICLES: HOW TO BALANCE FORCES

Ana F. Cunha$_P$[1], Mariana B. Oliveira[1], João F. Mano[1]

[1] *Department of Chemistry, CICECO – Aveiro Institute of Materials. University of Aveiro. Campus Universitário de Santiago. 3810-193 Aveiro, Portugal*

Corresponding author's email: afnc@ua.pt

Particle size in free-packed systems has been overlooked as a possibly relevant parameter to control cell response. We here explore the hypothesis that there is a lower limit of the traction force necessary for cells to adhere, effectively spread and move, which could be controlled by microsphere size. We aimed at understanding how cells sense and respond to those forces and how that drives cell fate, using a quasi-3D free-packed microparticle system in a liquid medium.

We used commercial polystyrene microspheres coated with type I collagen and assessed the adhesion and phenotype of human adipose tissue-derived mesenchymal stem cells (hASC). By focusing on different size ranges of microparticles - 14-20 µm, 38-45 µm, 85-105 µm -, we evaluated the metabolic activity, cell adhesion and morphology, besides focal adhesion formation after stipulated incubation timepoints (4 hours, 1, 3 or 4, 7 days).

Metabolic activity quantification suggested that initial cell adhesion to particles is not hampered in any microparticle size. Nonetheless, after 4 hours, microscopy data showed different cytoskeletal rearrangement profiles with intermediate and higher diameter microspheres presenting higher numbers of adhered cells with stretched actin fibers. Metabolic activity assessment corroborated that increasing microsphere diameters promoted cell proliferation over time (7 days), while detachment from smaller (~14 µm) microparticles explains the detected decline of observable cells and metabolic activity.

We hypothesize that higher and intermediate dimension spheres benefit long-term cell adhesion, whereas smaller microparticles may be an unsteady substrate for cell survival, due to the movement created by cells' pulling forces.

Abstract **#1489**

EVALUATION OF HUMAN BIOENGINEERED LAMELLAR CORNEAS GENERATED WITH FIBRIN-AGAROSE BIOMATERIALS

Miguel Alaminos$_P$[1], Carmen González-Gallardo[2], Óscar-Darío García-García[1], David Sánchez-Porras[1], Jesús Chato-Astrain[1], Cristina Blanco-Elices[1], Víctor Carriel[1], Antonio Campos[1], Fernando Campos[1], Miguel Ángel Martín-Piedra[1]

[1] *Tissue Engineering Group, Department of Histology, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain*

[2] *Division of Ophthalmology, Univ. Hospital San Cecilio, Granada, Spain*

Corresponding author's email: malaminos@ugr.es

BACKGROUND: Treatment of severely damaged corneas affected by trophic ulcers and other conditions is challenging. In this regard, a clinical trial to evaluate the clinical usefulness of a model of bioengineered lamellar human cornea generated as an advanced therapy medicinal product (ATMP) (1) is being currently implemented. The objective of this work is to evaluate the expression of relevant tissue molecules in these clinical-grade bioartificial corneas as compared with human native corneas.

METHODS: Human bioengineered corneas generated as ATMP were analyzed by immunohistochemistry using specific primary antibodies anti-cytokeratin and anti-plakoglobin and connexin, relevant cell-cell junction proteins in cornea.

RESULTS: The bioartificial cornea was biomimetic of the native cornea, although the differentiation level of the cornea epithelium was lower as compared to the human organ. Expression of the epithelial markers plakoglobin, connexin and cytokeratin was similar to control corneas.

CONCLUSIONS: These results imply that bioengineered human corneas are able to synthetize some of the proteins involved in human cornea differentiation and function and, therefore, fulfill the requirements for clinical use.

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Abstract **#1490**

BRIDGING THE GAP BETWEEN THE MECHANICAL AND METABOLIC ACTIVITY IN MSC DIFFERENTIATION

Ana San Felix$_P$[1], Sara Trujillo Muñoz[1], Matthew Dalby[1], Manuel Salmeron-Sanchez[1]
[1] *Centre for the Cellular Microenvironment (CeMi), University of Glasgow,*
Corresponding author's email: a.san-felix.1@research.gla.ac.uk

Cells are in constant communication with their surroundings and every information gathered from their vicinity is used to generate an ultimate response(1). To do that cells exert forces of different magnitude depending on how they sense the extracellular matrix(2). We hypothesise that every mechanical modification that cells undergo during their attachment, migration, proliferation and differentiation has a direct impact in their metabolic activity(3). This study aims to fill the existing gap between cell mechanical and metabolic activities, providing a better understanding on how microenvironmental cues can be used to control cell fate.

In this study, mesenchymal stem cells (MSCs) are seeded on full-length fibronectin(FN)-PEG hydrogels(4) with different stiffness and degradability. By using traction force microscopy (TFM), we observed that cells exert higher forces on degradable gels compared with non-degradable, and on stiffer gels compare with soft. When cytoskeleton contractility was inhibited, cells exerted lower forces on the surfaces. Looking at the mechanotransduction factor YAP, its nuclear location was higher on non-degradable surfaces compared with degradable ones. However, there were no significant differences when cytoskeleton contractility was inhibited using blebbistatin.

These experiments suggest that cell behaviour can be regulated by substrate's rigidity and degradability. In addition, cytoskeleton contractility has a great importance in force generation. It was interesting to see that YAP results differ from what we saw in TFM. Finally, based on these results, we are investigating how the cellular metabolic profile will change under these same properties of the environment.

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Abstract **#1491**

FUNCTIONAL MODIFIED COLLAGEN GEL PROMOTED THE RECOVERY OF MYOCARDIAL FUNCTION AFTER ISCHEMIA REPERFUSION INJURY

Tzuhsuan Chan[P][1]

[1] *Affiliated Drum Tower Hospital, Medical School of Nanjing University*

Corresponding author's email: zxzhan2018@sinano.ac.cn

Myocardial ischemia reperfusion injury happens at left anterior descending artery which branch from left coronary artery belongs to coronary artery disease (CAD). In China, CAD is the major cause of cardiovascular disease (CVD) with a proportion higher than 40%, while CVD is the leading cause of death in non-communicable diseases in the 2019 world health statistics. Angiogenesis therapies that decrease the death of cardiomyocytes by promoting the formation of new vessels restoring myocardial function are required for CAD patients' better quality of life.

Basic fibroblast growth factor (bFGF) is a well- known angiogenic stimulators which plays an important role in the angiogenesis process of migration, proliferation, and vascular tube formation directly. While stromal cell derived factor-1α(SDF-1α) stimulate the happening of angiogenesis by guiding artery cell's migration during collateral development. However, the combining effect of bFGF and SDF-1α on angiogenesis after ischemia reperfusion injury is unknown. Collagen gel is considered because of its well-known biocompatibility, low antigenicity, and its safety and feasibility, as the myocardium ECM is mostly comprised of collagen.

In this study, an injectable collagen gel modified with bFGF and SDF-1α was injected into the hearts of rats after induced ischemia reperfusion injury. Our results showed that the combined injectable collagen gel significantly promotes neo-vasculature forming and lessens fibrosis degree. In addition, it shows great enhancement in the functional restoration after heart ischemia reperfusion injury. Therefore, the combination of two factors, bFGF and SDF-1α, with injectable collagen gel provides a promising outcome after ischemia reperfusion heart injury.

Abstract **#1492**

NANOCOMPOSITE PIEZOELECTRIC SCAFFOLDS INCORPORATING LITHIUM NIOBATE FOR NEXT GENERATION COCHLEAR IMPLANT

Bahareh Azimi[1,2,3], Mariarita Candito[4], Alessandra Fusco[5], Mohammad Sajad Sorayani Bafqi[6], Claudio Ricci[1], Caterina Cristallini[7], Masud Latifi[6], Giovanna Donnarumma[5], Andrea Lazzeri[1], Laura Astolfi[4], Stefano Berrettini[8], Serena Danti[1,2,3]

[1] Dept. of Civil and Industrial Engineering, University of Pisa, Largo Lucio Lazzarino, 56126 Pisa, Italy

[2] Dept. of Civil and Environmental Engineering, Massachusetts Institute of Technology (MIT), Massachusetts Ave. 77, Cambridge, MA, USA

[3] National Interuniversity Consortium of Materials Science and Technology (INSTM), via G. Giusti 9, 50121 Firenze, Italy

[4] Bioacoustics Research Laboratory, Dept. of Neurosciences, University of Padua, via G. Orus, 2b, 35129 Padua, Italy

[5] Dept. of Experimental Medicine, University of Campania "Luigi Vanvitelli", via Santa Maria di Costantinopoli 16, 80138 Naples, Italy

[6] Dept. of Textile Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran

[7] Institutes for Chemical-Physical Processes, National Research Council (CNR-IPCF), via Moruzzi 1, 56126 Pisa, Italy

[8] Dept. of Surgical, Medical, Molecular Pathology and Emergency Medicine, University of Pisa, Largo Lucio Lazzarino, 56126 Pisa, Italy

Corresponding author's email: azimiaut@gmail.com

Sensorineural hearing loss is a pathology that affects the inner ear [1]. It is characterized by a lack of signal transduction, from the mechanic (fluid waves) to electric (depolarization of spiral ganglion neurons, while the nerve still maintains its function [2]. To date, the therapeutic option for patients suffering intense SNHL is the cochlear implant (CI), a high-tech electronic device replacing the entire cochlear function. Piezoelectric materials can play an important role in SNHL treatment by simply mimicking the function of the cochlear sensory epithelium. We aimed at developing significant advancements about piezoelectric biomaterials for cochlear implants as an innovative technology to aid hearing loss. Lithium niobate (LiNbO3), a piezoelectric ceramic, has been investigated as a potential candidate material for next-generation CIs. Lithium niobate nanoparticles resulted otocompatible with inner ear cells in vitro, had strong immunomodulatory activity and enhanced human defensin in epithelial cells, and also showed direct antibacterial activity against P. aeruginosa. Moreover, the electrospinning technique was used to produce LiNbO3-loaded polyvinylidene fluoride-trifluoro ethylene [P(VDF-TrFE)] composite fiber mesh. Aligned composite fibers containing 20% w/w% of LiNbO3 showed an enhanced piezoelectric response. These composite fibrous structures also supported human neural-like cell growth in vitro. Development of high-performance electroactive biomaterials via a combination of nanotechnology and tissue engineering opens new opportunities for cochlear stimulation and otoprotection, and would improve the quality of life of deaf people since they are cost-effective, simple, biomimetic, water and magneto-compatible.

Acknowledgements: NANOSPARKS project (MISTI, MIT-UNIPI program 2017).

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Abstract **#1494**

SUPRAMOLECULAR ASSEMBLY AND ANTIMICROBIAL PROPERTIES RELATIONSHIP OF HYBRID BIOPOLYMERS FROM ANTIMICROBIAL PEPTIDES AND ELASTIN-LIKE RECOMBINAMERS

Sergio Acosta[P][1], Zhou Ye[2], Conrado Aparicio[2], Arturo Ibañez-Fonseca[1], Matilde Alonso[1], Jose Carlos Rodriguez-Cabello[1]

[1] *Bioforge Lab, University of Valladolid (Spain)*

[2] *MDRCBB, University of Minnesota (USA)*

Corresponding author's email: sacosta@bioforge.uva.es

Elastin-like recombinamers (ELRs) have gained increasing interest because of their widespread application in drug delivery and tissue engineering.[1,2] Here, antimicrobial peptides (AMPs) have been co-produced with ELRs in a modular design in order to combine the antimicrobial potential of the AMPs with the stimuli-responsiveness of the ELRs for the development of antimicrobial and self-assembling biomaterials.

We investigated two different approaches: (i) L- and D-enantiomers of a designer AMP were incorporated in a low-fouling ELR[3] (AMP-ELR1). ELR1 remains unfolded at physiological conditions and a C-terminal grafting domain was incorporated. (ii) Self-assembling AMPs were cloned on the hydrophilic block of an amphiphilic ELR (AMP-ELR2) in order to assess their use as self-assembling domains to drive hierarchical organization of intrinsically-disordered proteins.

We developed an innovative and scalable method for the bioproduction of antimicrobial polypeptides. When AMP-ELRs were tethered on metal surfaces and the AMPs forced to be exposed (AMP-ELR1), ELR low-fouling activity seemed to converge synergistically with the antimicrobial properties of the AMP providing safe nanocoatings with strong antibiofilm properties.[4,5] In contrast, the ability of AMPs and ELRs to self-assemble in solution led to hierarchical architectures by way of a dual-assembly process (AMP-ELR2). First, the AMPs triggered the formation of nanofibers; then, the thermoresponsiveness of the ELR2 enabled assembly into fibrillar networks with potential application as molecular nanoreservoirs and physically crosslinked three-dimensional scaffolds for tissue engineering applications.[6] Our findings evidenced that the combination of AMPs and ELRs is a promising tool for improving the properties of the AMPs controlling their supramolecular assembly.

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Abstract **#1495**

MICROFLUIDIC SELF-ASSEMBLY OF CELL-LADEN MICROGELS INTO CLUSTERS, CHAINS AND ARRAYS: FROM ORGANOIDS TO VASCULATURE NETWORKS ENGINEERING.

Jan Guzowski[P][1], Katarzyna Rojek[1], Marco Costantini[1], Monika Ćwiklińska[1], Robert Buda[1]
[1] *Institute of Physical Chemistry of Polish Academy of Sciences*
Corresponding author's email: jguzowski@ichf.edu.pl

We report microfluidic generation of a variety of compartmentalized cell-laden hydrogel structures ranging from hydrogel bead-clusters and quasi-1D granular threads to 2D granular arrays and demonstrate that such types of tailored complex structures can be used to impose spatial cell-patterning in various types of biomaterials. We adopt the idea of capillary self-assembly of hydrogel beads inside a non-crosslinked hydrogel-precursor liquid layer, a method originally proposed over a decade ago by Du et al. [1]. Here, we propose a way of manipulating the capillary-arrested microbeads with unprecedented precision, throughput and reproducibility using microfluidics [2], which opens new perspectives in tissue engineering.
Our approach allows for directed self-assembly of hydrogel microbead clusters and we demonstrate possible applications of such structures in formulation of organoids such as artificial pancreatic islets or microtumors. We also demonstarte deposition of bead-chains and multi-chains, as well as perturbed quasi-1D structures at a substrate. The quasi-1D structures remain regular, yet stochastic; this allows encoding of the information about the encapsulated biomaterial and unique 'labeling' of the microbioreactors. We discuss potential applications of the technology in high-throughput drug screening.
Finally, we also report microfluidic assembly of endothelial-cell coated microbeads into regularly-spaced 2D arrays embedded in external fibrin matrix. The beads serve as angiogenic 'seeds': we observe outgrowth of capillaries, branching and finally anastomosis of microvessels coming from the neighboring beads indicating formation of a complex vascular network. We discuss the perspectives of the system in organ-on chip studies on vascular diseases.

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Abstract **#1496**

INLINE STIFFNESS TUNING OF MEW-GENERATED SCAFFOLDS VIA LASER PHOTOCROSSLINKING

Amit Chandrakar$_P$[1], Nadine Nottrodt[2], Tom Strudley[3], Lorenzo Moroni[1], Paul Wieringa[1]

[1] *Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University*

[2] *Fraunhofer-Institut für Lasertechnik ILT*

[3] *NKT Photonics*

Corresponding author's email: a.chandrakar@maastrichtuniversity.nl

Melt electrowriting (MEW) technique is a relatively new manufacturing technology used to fabricate scaffolds through the deposition of polymeric fibres according to a user-oriented design. Polymer melts, hydrogels and bioinks have been successfully spun via MEW, with both hydrogels or bioinks undergoing crosslinking by controlling the temperature at the nozzle section or by applying a post-process such as UV curing. In this work, we develop an inline photocrosslinking approach via laser irradiation within the nozzle section. Polyethylene glycol diacrylate (PEGDA) of Mw 3k and 6k are used as a material with two different photoinitiators, i.e. Irgacure and LAP. Melt rheology is performed under various conditions to determine pot life, processing temperature window and crosslinking parameters. A conceptual model is described based on rheology date to facilitate the choice and design of a laser module and finally integrated into custom-made MEW system. MEW of the PEGDA is successfully done at a melting temperature of 60°C and by crosslinking with a laser. While changing the laser intensity, the filament stiffness is successfully tuned inline, making it possible to control the stiffness of the filament at the desired time/location. Due to greater control over stiffness compared to other methods of crosslinking, this technique is useful for creating anisotropic scaffolds without changing the design patterns. Mechanical tests have been performed to show the different anisotropic behaviour of identical square pattern scaffolds.

Abstract **#1498**

POLYVINYL ALCOHOL/GELATIN SPONGES FOR AURICULAR CARTILAGE ENGINEERING

Bahareh Azimi[P][1,2], Marta Feula[3], Mario Milazzo[4], Arti Ahluwalia[3], Stefano Berrettini[5], Serena Danti[1,2,4]

[1] *National Interuniversity Consortium of Materials Science and Technology (INSTM), via G. Giusti 9, 50121 Firenze, Italy*

[2] *Dept. of Civil and Industrial Engineering, University of Pisa, Largo Lucio Lazzarino, 56126 Pisa, Italy*

[3] *Research Center "E. Piaggio", University of Pisa, 56122 Pisa, Italy*

[4] *Dept. of Civil and Environmental Engineering, Massachusetts Institute of Technology (MIT), Massachusetts Ave. 77, Cambridge, MA, USA*

[5] *Dept. of Surgical, Medical, Molecular Pathology and Emergency Medicine, University of Pisa, Largo Lucio Lazzarino, 56126 Pisa, Italy*

Corresponding author's email: azimiaut@gmail.com

Reconstructing auricle resulting from trauma, neoplasm, or congenital defects is one of the most challenging and diverse tasks in aesthetic and reconstructive surgery due to the highly complex three-dimensional anatomy of the outer ear [1]. We aimed at providing alternatives to overcome the shortcomings of the standard surgical reconstructive procedures using tissue engineering. Emulsion and freeze-drying were used to produce poly(vinyl alcohol)/gelatin (PVA/G) sponges at different weight ratios (100/0 - 50/50 w/w%), which were cross-linked by exposure to glutaraldehyde vapors. PVA/G sponges with highly round-shaped interconnected pores, highly swelling capacity (> 200%), and an essentially elastic mechanical behaviour were obtained. Different culture conditions were applied to obtain elastic cartilage: , undifferentiated versus chondrogenic pre-differentiated bone marrow derived human mesenchymal stromal cells (hMSCs), commercial versus hand-made chondrogenic differentiation medium and static versus dynamic culture [i.e. ultrasound (US) or bioreactor stimulation] were used. After three weeks, the constructs were analyzed via immunohistochemistry (IHC). Intense glycosaminoglycan, glycoprotein and collagen syntheses by hMSCs were most frequently observed using the commercial medium, while round morphology was observed when pre-differentiated hMSCs were seeded. The application of US stimulation during the culture on cell/scaffold constructs enhanced extracellular matrix deposition and led to 30% higher collagen type II expression at gene level. However, at protein level, collagen type II, aggrecan and elastin formation were observed via IHC only by using bioreactor culture. Results showed that 70/30 (w/w%) PVA/G sponge is a suitable scaffold for auricle reconstruction.

Acknowledgements: AURICULAE Project, funded by Stem Cells & Life Foundation, Padova, Italy.

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Abstract **#1499**

UNIAXIAL CYCLIC STRAIN TO RESTORE THE ANISOTROPY OF CARDIAC CELLS AND ECM IN 2D AND 3D IN VITRO MODELS OF MYOCARDIAL INFARCTION

Dylan Mostert[P][1,2], Ignasi Jorba[1,2], Nicholas Kurniawan[1,2], Carlijn Bouten[1,2]

[1] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands*

[2] *Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, the Netherlands*

Corresponding author's email: d.mostert@tue.nl

The human myocardium is a mechanically active tissue typified by its anisotropic organization of cells and extracellular matrix. Upon injury, the myocardium undergoes dramatic alterations, resulting in disruption of anisotropy and loss of coordinated contraction. Moreover, loss of anisotropic organization hampers the differentiation, matrix production, and mechanotransduction of resident and newly injected cardiac cells. Therefore, restoring the anisotropic organization in the injured myocardium could greatly benefit myocardial regeneration.

In this project, we studied the effect of mechanical and structural cues, inspired by myocardial biology, on the organization of cardiac cells. We showed that uniaxial cyclic strain, mimicking the local deformation of cardiac beating, led to anisotropic organization of cardiac fibroblasts (cFBs), but not of cardiomyocytes (hiPSC-CMs). Next, we reconstructed the cellular compositions of normal and pathological myocardium using co-cultures with varying cell ratios. Surprisingly, contrary to the response of the hiPSC-CM monoculture, the co-cultures adopted an anisotropic organization under uniaxial cyclic strain, regardless of the co-culture composition. These data suggest that the mechanoresponsiveness of cFBs may be critical in determining myocardial tissue structure and function.

To further investigate the relevance of these mechanical and structural cues in vivo, we developed 3D myocardial micro-tissues, consisting of cell-laden collagen I/Matrigel constructs within flexible micropillars. Using this model, we successfully imposed mechanical constraints to tune the degree of microtissue organization and allow measurement of tissue contraction. We will discuss how uniaxial cyclic strain can be used to induce anisotropy in a 3D myocardial micro-tissue and how this effects tissue contraction and function.

Keywords

Cellular Organization; Cyclic strain ; Cellular Mechanosensing

Abstract **#1501**

CERIUM DOPED MESOPOROUS NANOPARTICLES FOR DRUG DELIVERY OF ARTEMISININ

Ioannis Tsamesidis[P][1], Dimitrios Gkiliopoulos[1], Georgia K. Pouroutzidou[1], Anna Theocharidou[1], Karine Reybier[2], Pierre Perio[2], Konstantinos Triantafyllidis[1], Konstantinos M. Paraskevopoulos[1], Eleana Kontonasaki[1]

[1] *Aristotle University of Thessaloniki, Greece*

[2] *University of Toulouse, France*

Corresponding author's email: kont@dent.auth.gr

Mesoporous nanoparticles (MSNs) constitute promising carriers for the adsorption and local control release of biological active agents that promote bone regeneration. Doping MSNs with cerium ions could contribute to control over bacterial contamination and enhance the capability of vascularization, eliminating the chance of bone loss1. The aim of this study was the evaluation of the antioxidant agent artemisinin2 loading/release profiles, biocompatibility and hemolytic activity of different Ce-doped MSNs (1, 2.5 and 5%). MCM-41 mesoporous nanoparticles were synthesized via sol-gel and had either neat siliceous or metal (Ca and Ce) substituted walls. X-Ray Diffraction analysis and N2 porosimetey were used to determine their structural characteristics. Their drug loading and release profiles were assessed with Liquid Chromatography–Mass Spectrometry (LC-MS) and their cell viability with the MTT assay on human periodontal ligament cells (hPDLCs). Their hemolytic activity was evaluated in contact with human red blood cells at various concentrations (12.5-500μg/ml). A high loading capacity was recorded for all Ce-doped MSNs (73-85%) that was higher compared to pure silica (64%). Artemisinin release was verified after sonication for 40 min, without significant differences among MSNs. At low concentrations MSNs presented no hemolytic activity, while at high concentrations the lowest hemolytic activity was recorded for the MSNs with the highest amount of cerium. The 1 and 2.5% Ce-doped MSNs significantly promoted mitochondrial activity and proliferation of hPDLCs. In conclusion, cerium can be incorporated in MSNs to improve their performance in terms of biocompatibility, hemolytic activity and loading of artemisinin.

Keywords

Cerium doped mesoporous nanoparticles; Artemisinin; Human periodontal ligament cells

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Abstract **#1502**

CHONDROPROTECTIVE EFFECTS OF IBUPROFEN-LOADED PLGA NANOPARTICLES IN 3D HUMAN CHONDROCYTE CULTURES UNDER A PRO-INFLAMMATORY STIMULUS

Cecília Juliana Alves[P][1,2], Beatriz Sousa[1,2,3], Marina Couto[1,2], Daniela P Vasconcelos[1,2], Catarina Pereira[1,2], Bruno Sarmento[1,2,4], Raquel Gonçalves[1,2,5], Meriem Lamghari[1,2,5]

[1] *I3S- Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal*

[2] *INEB- Instituto de Engenharia Biomédica, Universidade do Porto, Portugal*

[3] *FEUP- Faculdade de Engenharia, Universidade do Porto, Portugal*

[4] *CESPU- Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal*

[5] *ICBAS- Instituto Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal*

Corresponding author's email: cjmalves@ineb.up.pt

Traumatic chondral lesions are common in the young and active population, and if left untreated can evolve towards osteoarthritis. The inflammatory response initiated as a consequence of tissue damage imposes a major challenge in cartilage repair. The released pro-inflammatory factors induce a shift in chondrocyte activity towards catabolism and promote further tissue degradation (1-2). Several anti-inflammatory drugs have been successfully tested (2), and we propose ibuprofen-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles as a strategy to locally deliver an anti-inflammatory treatment.

Here, we aimed to evaluate the chondroprotective and chondrogenic effects of ibuprofen-loaded PLGA nanoparticles. To achieve this, human chondrocytes were cultured as 3D pellets, submitted to a pro-inflammatory environment (stimulation with 100ng/mL of IL-1β), and treated with PLGA nanoparticles loaded with 15 or 30 μg/mL of ibuprofen. Analyses were performed 3 and 7 days after nanoparticles addition.

Our results show that 3 and 7 days treatment with ibuprofen-loaded nanoparticles (15 or 30 μg/mL of ibuprofen) leads to a decrease in both IL-1β protein release and metalloproteases mRNA expression (MMP1 and MMP8) by human chondrocyte pellets cultured under a pro-inflammatory stimulus. Moreover, under similar conditions, 3 days treatment with these nanoparticles induced an increased production of extracellular matrix (ECM) components (collagen type II and aggrecan). Altogether the obtained data indicate that treatment with ibuprofen-loaded PLGA nanoparticles mitigates chondrocytes' pro-inflammatory response and ECM degradation activity induced in an inflammation scenario. Therefore, this is a promising strategy to overcome catabolic effects of post-traumatic inflammation on cartilage.

Study supported by the EU project RESTORE (REF:814558).

Keywords

Inflammation

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Abstract **#1503**

TOWARDS ROBOTICS-DRIVEN MANUFACTURING OF SPHEROID-BASED SKELETAL IMPLANTS

Isaak Decoene$_P$[1], Gabriella Nilsson Hall[1], Rodrigo Furtado Madeiro de Costa[2], Inge Van Hoven[1], Samuel Ribeiro[1], Angela Pastore[1], Jens Eberhardt[3], Catherine Verfaillie[2], Liesbet Geris[1], Frank P. Luyten[1], Ioannis Papantoniou[1]

[1] Prometheus Division of Skeletal Tissue Engineering, KU Leuven, O&N1, Herestraat 49, PB 813, 3000 Leuven, Belgium

[2] Department of Development and Regeneration, Stem Cell Biology and Embryology, KU Leuven, Leuven, Belgium

[3] ALS automated lab solutions, Jena, Germany

Corresponding author's email: isaak.decoene@kuleuven.be

For a viable and compliant clinical translation of tissue engineered products, the adoption of automated technologies has been acknowledged as a prerequisite. Recently, the use of chondrogenic microtissue and organoid assemblies has shown promising results in long-bone defect regeneration through endochondral ossification[1]. Hence, automated biomanufacturing technologies able to culture and handle these tissue building blocks are of great interest. Here, we present an automation strategy through the use of different robotics for (i) media change during differentiation and (ii) image-based picking of microtissues for enabling spheroid-specific QC.

Media change of microtissues in microwell platforms requires controlled pipetting to avoid microtissue displacement and suspension leading to uncontrolled fusion. A first design of experiment (DoE) revealed that dispensing speed and its interaction with pipetting needle depth during dispensing affected microtissue displacement the most. Conversely, aspiration speed within the predefined ranges had less impact. A second DoE was created to improve protocol parameters for microtissues of different sizes (250/1000 cells). Smaller microtissues moved more than large, especially at higher aspiration speeds. Subsequently, the automatic cell-screening and -picking system CellCelector™ was used to select and transfer single spheroids in a controlled manner. Spheroids were automatically selected based on the presence of only 1 spheroid/well and/or size via image-based analysis. The aspirated spheroid was subsequently deposited in a pre-defined receiver-microwell for further analysis.

We demonstrate the development of an integrated bioprocess for culturing and manipulation of cartilaginous spheroids. We anticipate the progressive substitution of manual operations with automated solutions for manufacturing of microtissue-based living implants.

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Abstract **#1504**

COMPLEX 3D VASCULARIZED ORGANOTYPIC MODELS MIMICKING MUSCOLOSKELETAL PATHOLOGICAL ENVIRONMENTS

Simone Bersini[1,2], Chiara Arrigoni[1], Maria Vittoria Colombo P[1,3], Christian Candrian[1], Matteo Moretti[1,2]

[1] *Regenerative Medicine Technologies Lab, Ente Ospedaliero Cantonale, Lugano (CH)*

[2] *Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milano (IT)*

[3] *Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta", Politecnico di Milano (IT)*

Corresponding author's email: mariavittoria.colombo@polimi.it

Several diseases affecting the musculoskeletal compartment (e.g. bone tumors, muscle fibrosis) involve the interplay among tissue-specific cells, vascular cells and extracellular matrix [1]. Currently available models are too simplified to address such a complex scenario and alternative systems able to recapitulate the native human tissue complexity are required.To overcome limitations of microfluidic models (e.g. limited tissue thickness, availability of biological material) we biofabricated mm-scaled models of bone and muscle tissues which are compatible with proteomics and gene expression analyses. Bone models included osteoblasts, osteoclasts, vascular cells and macrophages in a fibrin matrix with or w/o hydroxyapatite nanoparticles. The muscle model embedded aligned human muscle fibers wrapped by endomysium and surrounded by vascular network. To analyze the behavior of healthy and pathological cells we combined immunofluorescence, flow cytometry and qPCR analyses. Exploiting microfluidic models we reproduced the higher extravasation rate of breast cancer cells (bCCs) in the bone compared to the muscle model [2]. This increased bCC affinity towards the bone was also demonstrated in the mm-scale models. We thus exploited our bone model as drug screening platform showing that doxorubicin and rapamycin reduced bCC proliferation while inducing vascular damage. The mm-scale muscle was evaluated as fibrosis model employing diseased human fibroblasts, showing its ability in replicating typical fibrosis features compared to standard 2D models [3].The generation of complex biological environments in our models allowed to replicate disease hallmarks and to investigate the effects of drugs more reliably than current standard models.

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Abstract **#1505**

THE USE OF AN ORBITAL SHAKING SYSTEM FOR OPTIMISING CELL CULTURE IN CARTILAGE TISSUE ENGINEERING

Thomas Jovic[P][1,2], Feihu Zhou[3], Shareen Doak[3], Iain Whitaker[1,2]

[1] *Reconstructive Surgery & Regenerative Medicine Research Group, Swansea University, UK*

[2] *Welsh Centre for Burns and Plastic Surgery, Morriston Hospital, Swansea, UK*

[3] *Swansea University*

Corresponding author's email: thjovic@doctors.org.uk

INTRODUCTION: Cartilage is responsive to mechanical stimulation, with the potential for exploitation in tissue engineering for reconstructive surgery. The aim of this study was to determine the role of dynamic culture conditions on cell growth and chondrogenesis independent of mechanical stimulation through the use of an orbital shaking system.

METHODS: Human nasal septal cartilage was acquired from septal surgery. Human nasal chondrocytes were seeded into 100ul nanocellulose and alginate hydrogels, crosslinked with calcium chloride and incubated at 37oC for 14 days in either static or dynamic conditions (orbital plate shaker, 500 rpm). Cell growth and chondrogenesis was confirmed using quantitative polymerase chain reaction, histological stains of cartilage extracellular matrix and cell proliferation assays. Relative gene expression was compared between static and dynamic groups using unpaired t-tests. Computational analysis was used to determine the degree of shear stress experienced by cells in the biomaterial in the orbital shaking conditions.

RESULTS: In nasal septal chondroprogenitor cell populations, dynamic conditions induced marked increases in the gene expression of chondrogenic genes COL2 (7.28-fold increase, $p<0.001$), aggrecan (10.26-fold increase, $p=0.001$) and SOX9 (2.34-fold, $p=0.002$). Histological analysis demonstrated increased matrix formation and superior biomaterial integrity in dynamic conditions. Computational modelling confirmed the shear stress exerted by orbital shaking was inadequate to directly affect chondrogenesis.

CONCLUSIONS: The use of an orbital shaking system exerts biologically relevant effects on human nasal chondrocytes independently of mechanical stimulation. We postulate this mechanism is through enhancing growth media perfusion and has implications for in vitro cartilage tissue engineering for reconstructive surgery.

Abstract **#1506**

PREPARATION OF BIOCOMPATIBLE OLIGOMERS

Cordula Hege[P][1], Amy Stimpson[1], Chris Fox[2], Derek Irvine[1]

[1] *University of Nottingham*

[2] *IDRI*

Corresponding author's email: Cordula.Hege@nottingham.ac.uk

Oligomers have potential application for printing and as adjuvants for vaccines. Preparing oligomers is especially worthwhile for stereolithography. So one gets materials in the needed viscosity range. Oligomers are favourable as adjuvants for vaccines, as they facilitate renal clearance. Methacrylic polymers known to be widely tolerated in vivo, so they are widely used in biology and medicine. So, to prepare the oligomers mainly methacrylic monomers were used.
The oligomers were prepared by catalytic chain transfer polymerisation (CCTP). Cobalt macrocycles are effective and mostly used catalysts for the CCTP. This is disadvantageous for medical applications, as cobalt is toxic. Iron has higher biocompatibility and is also abundant in nature, so it is additionally more sustainable. The catalysts were prepared in situ. For that iron bromide and dimethyl glyoxime or diphenyl glyoxime were added as ligands. Different catalyst concentrations were used. The reactions were made in bulk and with toluene as solvent.
The reaction conditions and monomers influenced the level of control. For reactions using diphenyl glyoxime as ligand it was preferable to prepare the oligomers in bulk. In contrast to that dimethyl glyoxime as ligand could control reaction better in solvent. To control the reactions higher concentration of iron catalysts were needed than in case of cobalt catalysts.
Iron catalysts are a possible replacement for cobalt catalysts for the CCTP. This allows to prepare reproducible, biocompatible oligomers. Further work will be done to optimise the conditions.

Abstract **#1507**

THE POTENTIAL OF HIPSC AND HIPSC - CARDIOMYOCYTES AS NOVEL THERAPEUTIC EV SOURCES FOR CARDIAC REGENERATION

Ana Filipa Louro[1,2], Marta Oliveira[1], Patrícia Gomes-Alves[1,2], Paula Alves[1,2], Margarida Serra[1,2]

[1] *IBET - Instituto de Biologia Experimental e Tecnológica*

[2] *ITQB - Instituto de Tecnologica Química e Biológica*

Corresponding author's email: ana.louro@ibet.pt

Studies on the cardiac repair potential of Extracellular Vesicles (EV) traditionally use mesenchymal or cardiac progenitor cell-derived EV [1,2], overlooking the potential of human induced pluripotent stem cells (hiPSC) and hiPSC-derived cardiomyocytes (hiPSC-CM) as alternative EV sources.

In this study, we characterized EV derived from key stages of the hiPSC-CM differentiation and maturation, i.e. from hiPSC (hiPSC-EV), cardiac progenitors (CPC-EV), immature (CMi-EV) and mature (CMm-EV) cardiomyocytes, with the goal of studying their potential role as therapeutics, and whether their yield and function was influenced by the state of their parent cell.

Two hiPSC lines were differentiated into hiPSC-CM and cultured as 3D spheroids in a fatty acid supplemented medium to improve CM maturation [3,4]. EV were isolated using density separation and characterized in terms of yield, particle size distribution, and bioactivity. EV yield varied along CM differentiation stages. Specific EV markers (CD63, CD81, TSG101) were present and the typical cup-shaped morphology was confirmed by TEM analysis.

Bioactivity assays with human umbilical vein endothelial cells (HUVECs) showed that uptake of PKH26-labelled EV could be blocked by dynasore. Increased migration was observed in HUVECs treated both with hiPSC and cardiomyocyte-derived EV, but angiogenic properties were only found for hiPSC-EV (fold change of 11.2 ± 4.59 in total segment length vs. control, $p<0.001$).

Ongoing work will elucidate the differences in the small RNA cargo and identify the regenerative functions associated with EV from each cell source.

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Abstract **#1508**

INVESTIGATING OSTEO-ARTICULAR DISEASES THROUGH MICROPHYSIOLOGICAL BONE AND JOINT MODELS

Matteo Moretti[P][1,2]

[1] *Ente Ospedaliero Cantonale*

[2] *IRCCS Istituto Ortopedico Galeazzi*

Corresponding author's email: Matteo.Moretti@eoc.ch

Microfluidic models can be precious tools in orthopedics, to study diseases such as bone metastases but also for osteoarthritis (OA), in which extravasation of cancer cells and immune cells is involved. Thus, in our lab we generated microfluidic models of bone, muscle and joint tissues, to study the preferential extravasation of cancer cells into bone and of immune cells into the synovium. Bone, muscle or synovial cells together with vascular cells were embedded in 3D fibrin matrices, generating tissue-specific compartments with perfusable microvascular networks. In joint models, a channel with chondrocyte in fibrin and a channel with synovial fluid were also present. Our models demonstrated how muscle-secreted adenosine and inhibition of key phosphorylation events of focal adhesion proteins impaired the process of cancer cell extravasation. Furthermore, we showed that the presence of blood cells and the features of surrounding tissue environment influenced the process. In the joint models, the addition of OA synovial fluid or inflammatory cytokines increased endothelium activation and monocyte extravasation. Furthermore, inflammation modulated MMPs expression and angiogenesis, whilst the injection of bMSCs and ASCs modified cellular morphology and secretion of inflammatory cytokines. In conclusion, the use of microfluidic models of bone and muscle tissues allowed to replicate the mechanism of preferential metastatization of breast cancer to bone, indicating possible mechanisms involved. On the other hand, the generation of a joint-on-a-chip model allowed to study inflammatory processes in OA and to simulate the administration of injective therapies to OA patients, evaluating their effects on joint tissues.

Abstract **#1509**

TOWARDS THE DEVELOPMENT OF HUMAN-BASED BONE ORGANOIDS FOR PERSONALIZED MEDICINE

Anke de Leeuw$_P$[1], Ben Kodiyan[1], Jianhua Zhang[1], Pei Jin Lim[2], Matthias Rüger[3], Marianne Rohrbach[2], Cecilia Giunta[2], Ralph Müller[1], Marina Rubert[1]

[1] *Institute for Biomechanics, ETH Zurich, Zurich, Switzerland*

[2] *Connective Tissue Unit, Division of Metabolism and Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland*

[3] *Department of Pediatric Orthopaedics and Traumatology, University Children's Hospital Zurich, Zurich, Switzerland*

Corresponding author's email: marina.rubert@hest.ethz.ch

Treatments for bone diseases that specifically target the individual's bone phenotype do not yet exist. Development of a human 3D-bioprinted bone model using patient-derived bone cells may represent an attractive approach to further our understanding of the pathomechanisms behind the genetic heterogeneity of bone diseases offering a platform to explore personalized therapies. However, tissue harvesting, cell isolation procedures and culture conditions influence the osteogenic potential of cells. Here, the influence of media compositions with (+) or without (-) bFGF and NEAA on osteogenic gene expression was investigated in patient-derived osteoblasts. Enhanced growth rates and upregulation of osteogenic markers (BGLAP and E11) similar to bone biopsies were only observed in cells cultured in medium +bFGF +NEAA. 3D bioprinting of patient cells expanded under these conditions had high cell viability (>90%). Similar to bone biopsies, PHEX (early osteocyte-related gene) was upregulated in organoids compared to 2D patient cells. Sirius red staining showed organoids were able to produce a collagen I matrix and micro-CT images revealed an average mineral volume of 53.6 ± 4.3 mm3 after 42 day-culture in osteogenic media. Osteocalcin immunohistochemistry staining indicated the presence of mature osteoblasts. Fluorescence imaging revealed osteocyte-like cell connections, reminiscent of the lacunar-canalicular network of bone. We have established the culture conditions that support the formation of a mineralized bone organoid with an osteoblastic and early osteocytic phenotype similar to in vivo bone tissues. In the future, these bone organoids may open new possibilities for personalized medicine to study and identify potential therapeutic targets.

Abstract **#1510**

INVESTIGATION OF THE EFFECTS OF PLATELET-RICH PLASMA AND MATRIDERM IN RECURRENT CARPAL TUNNEL SYNDROME

Mehmet Bozkurt[P][1], Burak Ergün Tatar[1], Percin Karakol[1], Melihcan Sezgic[1], Caner Gelbal[1]
[1] *Bagcılar Training and Research Hospital Department of Plastic Surgery, Istanbul,Turkey*
Corresponding author's email: drmbozkurt@yahoo.com

Background: Surgery is the most common treatment of carpal tunnel syndrome (CTS). The increased recurrence of clinical and electrophysiological findings and absences of their regressions were reported in some cases after surgery. In this study, we aimed to see the effect of Matriderm and platelet-rich plasma (PRP) on the clinical and electrophysiological findings in patients with secondary CTS.
Methods: Seven patients with secondary CTS were included in our study. PRP and Matriderm were applied to the patients as an adjuvant to the surgery. Patients were administered Q-DASH and Visual Analogue Scale (VAS) and underwent electrophysiological tests such as; compound motor action potential (CMAP), motor nerve conduction latency (ms), motor nerve conduction velocity (m/s), sensory nerve action potential (SNAP), sensory nerve conduction latency (ms), and sensory nerve conduction velocity (m/s) in the preoperative period and the postoperative twelve months both after primer and revision operation.
Results: After revision surgery: No significant difference was found in terms of CMAP(compound motor action potential). ($p>0.05$) However, Q-DASH, Visual Analogue Scale (VAS), and other electrophysiological parameter values varied significantly different from preoperative and primary surgery values.($p<0.05$)
Conclusions: As a result, it can be concluded that the use of Matriderm and PRP together in the surgery for CTS treatment increases neural regeneration and reduces scar formation and the recurrence of symptoms, thus improving the recovery after revision of the surgery.

Keywords
Carpal Tunnel Syndrome; Matriderm; Platelet-Rich Plasma

Abstract **#1511**

LINKING FUNCTIONALITY OF BONE-FORMING MICROTISSUES TO SECRETED PROTEOME: TOWARDS PREDICTIVE POTENCY MONITORING

Isaak Decoene$_P$[1], Mohamed Belal Hamed[2], Gabriella Nilsson Hall[1], Frank P. Luyten[1], Anastassios Economou[2], Ioannis Papantoniou[1,3]

[1] *Prometheus Division of Skeletal Tissue Engineering, KU Leuven, O&N1, Herestraat 49, PB 813, 3000 Leuven, Belgium*

[2] *Department of Microbiology and Immunology, Rega Institute, Laboratory of Molecular Bacteriology, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium*

[3] *Institute of Chemical Engineering Sciences, Foundation for Research and Technology—Hellas (FORTH), Greece*

Corresponding author's email: isaak.decoene@kuleuven.be

For a successful translation of tissue engineered implants to the clinic, non-destructive monitoring of differentiation status of cells in microtissues is of paramount importance.

We aimed at tracking the secretome of human periosteum derived microtissues during in vitro chondrogenic differentiation in relation to their in vivo endochondral bone forming potency. For that, we cultured microtissues from six donors in a serum-free chondrogenic medium which enabled proteomics characterisation of the supernatant. Microtissues were monitored with brightfield microscopy, and samples were taken for DNA quantification. Additionally, conditioned media was collected for high-throughput LC-MS/MS secretome identification. Microtissues showed an increased expression of chondrogenic markers (SOX9, COL2, COL10). On average, DNA content increased in the first week (158% of the starting amount), followed by a decrease to 92%. After 3 weeks of culture, 600 microtissues were fused over 24 hours and implanted ectopically in nude mice for 4 weeks. Bone ossicles containing bone marrow compartments were found for 4 out of 6 donors as shown by nano-computed tomography. Conditioned medium from the first donor contained 731 proteins, of which 278 are secreted. Of these, we identified 10 proteins that were significantly elevated between week 1 and 3.

In conclusion, we carried out a series of orthogonal quality characterisation studies and used high-sensitivity proteomics for mapping the secretome during chondrogenic differentiation. This is promising for the identification of biomarkers that will enable the implementation of quality-by-design strategies by predictively controlling the microtissue differentiation processes in vitro as to ensure skeletal defect regeneration upon implantation.

Abstract **#1512**

DEVELOPMENT OF A 3D MODEL OF OSTEOSARCOMA TO CHALLENGE THE EFFICIENCY OF PLASMA-CONDITIONED LIQUIDS

Cristina Canal[1], Juan Tornin[1], Miguel Mateu[1], Xavi Solé-Martí[1], Maria-Pau Ginebra[1]

[1] *Universitat Politècnica de Catalunya*

Corresponding author's email: cristina.canal@upc.edu

The use of Cold Atmospheric Plasma in oncology is an emerging field due to its huge potential as novel anti-cancer therapy [1]. Plasma chemistry leads to the generation of an abundance of reactive species (RONS) which are suspected to play a key role in selective cancer cell death without damaging surrounding healthy tissues. Such effects have also been observed in plasma conditioned liquids (PCL), opening the door for minimally invasive therapies. However, the beneficial effects of plasma and of plasma-treated liquids for treating cancer have mostly been demonstrated in 2-dimensional cultures of cells [1, 2, 3], which do not mimic the complexity of the 3-dimensional (3D) tumor microenvironment.

To evaluate the effects of plasma in a relevant context o, we developed a 3D tissue-engineered model of osteosarcoma using a bone-like scaffold made of collagen type I and hydroxyapatite nanoparticles. Different human osteosarcoma cells were cultured within the scaffold showing high capacity to infiltrate and proliferate and to exhibit osteomimicry in vitro. Significantly different functional behaviors were observed between monolayer and 3D cultures when treated with PCL. Our data reveal that the 3D environment protects cells from PCL-induced lethality by scavenging and diminishing the amount of reactive oxygen and nitrogen species generated by plasmas, but also favours the stemness phenotype of osteosarcoma cells, demonstrating for the first time the negative effect of PCL on cancer stem-like cell subpopulations in a 3D biomimetic cancer model. These findings may allow to suitably re-focus research on plasma-based therapies in future.

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Abstract **#1513**

PHENOTYPING OF CORNEAL DERIVED MESENCHYMAL STEM CELLS (CMSCS) FOLLOWING ADDITION OF PROINFLAMMATORY STIMULI

Lydia Beeken[P][1], Grazziela Figueredo[2], Cameron Alexander[3], Felicity Rose[3], Laura E. Sidney[1]

[1] *Academic Ophthalmology, Division of Clinical Neuroscience, University of Nottingham, Nottingham, UK*

[2] *Advance Data Analytics Centre, School of Computer Science, University of Nottingham, Nottingham, UK*

[3] *School of Pharmacy, University of Nottingham, Nottingham, UK*

Corresponding author's email: lydia.beeken@nottingham.ac.uk

Corneal mesenchymal stem cells (CMSCs) have demonstrated great anti-inflammatory potency, making them ideal candidates as therapeutic agents for ocular surface inflammatory disorders. Previous phenotypic analysis has been used to demonstrate the expression of classical MSC markers by the cells, however phenotypic profiling has not been carried out. In this study, we aimed to identify key phenotypic markers of CMSCs and compare these with previously identified MSC markers from alternative sources. Additionally, we aimed to develop an understanding of any CMSC phenotypic and behavioral changes following activation with a pro-inflammatory inflammatory cytokine 'cocktail'.

The BD Lyoplate™ Human Cell Surface Marker Screening Panel was utilised with flow cytometry to assess CMSC expression of 242 markers of unstimulated cells, cells stimulated with pro-inflammatory cytokines and stimulated cells with a recovery period. Cell behaviour, viability and morphology was also assessed.

Following activation with the cytokine 'cocktail', a subpopulation of cells remained viable, with reversible inhibition of proliferation. The remaining population of cells also demonstrated increased potential for release of anti-inflammatory factors compared to unstimulated CMSCs. CMSCs displayed a similar phenotypic profile to bone marrow and umbilical cord derived MSCs depicted in the literature [1], with CD140a, CD49d, CD26 and CD95 identified as specific to CMSCs. Furthermore, significant phenotypic changes were observed in the cells following stimulation and recovery.

Here, we have developed a phenotypic and behavioural profile of CMSCs, which is vital for safe and effective translation of the cells as a clinical cell therapy.

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Abstract **#1514**

SAFETY, OSTEOINDUCTIVE AND OSTEOINTEGRATIVE PROPERTIES OF TITANIUM CERAMIC SCAFFOLDS

Min Hwei Ng[P][1], Lohashenpahan Shanmuganantha[2], Azmi Baharudin[3], Abu Bakar Sulong[4], Roslinda Shamsudin[5], Muhammad Azmi Abdul Hamid[5]

[1] *Tissue Engineering Centre, Faculty of Medicine, Universiti Kebangsaan Malaysi*

[2] *Tissue Engineering Centre, Faculty of Medicine, Universiti Kebangsaan Malaysia*

[3] *Department of Orthopaedic and Traumatology, Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia*

[4] *Department of Mechanical Engineering, Faculty of Engineering & Built, Universiti Kebangsaan Malaysia*

[5] *School of Applied Physics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia*

Corresponding author's email: angela@ppukm.ukm.edu.my

Titanium, a popular material used for bone implants due to its bioinert and strength. The poor cell attachment and osteointegration properties, and stress shielding effect associated with its motivated the exploration of the titanium-ceramic composite. Titanium-ceramic composite has enhanced bioactivity and a mechanical property closer to bone. This study was conducted to investigate the affinity of mesenchymal stem cells towards both titanium-hydroxyapatite and titanium wollastonite. Surface characteristics through scanning electron microscopy show that both the composite materials exhibit rough surfaces which portents good cell attachment. This has been also confirmed through the use of atomic force microscopy to give a quantitative assessment of the materials concerned. Titanium ceramic showed enhanced mesenchymal stem cell attachment, cell proliferation, and osteogenic gene expressions compared to titanium. MSCs seeded on titanium ceramic maintains their immunomodulatory property in terms of activated T-cell suppression. Compared to titanium-hydroxyapatite, titanium-wollastonite showed greater bioactivity. Wollastonite is lower in cost compared to hydroxyapatite and can be derived from natural resources. Hence, the prospect of titanium-wollastonite as a scaffold for tissue engineering scaffold or bone implant material should be explored further. Finally, the safety and osteointegration properties of titanium vs titanium ceramic in vivo are currently being investigated.

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Keywords

Titanium ceramic; Osteointegration; Osteoinduction

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Abstract **#1515**

DEVELOPMENT, AND PRECLINICAL EVALUATION, OF A NOVEL, BIOINSPIRED BIOMATERIAL, FOR USE AS A CALCIFIED TISSUE ADHESIVE.

Michael Pujari-Palmer[P][1], Philip Procter[1], Gry Hulsart-Billstrom[2,3], Gerard Insley[1], Sune Larsson[2], Hakan Engqvist[1]

[1] *Applied Material Science, Department of Engineering, Uppsala University, Uppsala, Sweden*

[2] *Department of Surgical Sciences, Faculty of Medicine and Pharmacy, Uppsala University, Uppsala, Sweden*

[3] *Preclinical PET-MRI Platform, Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden*

Corresponding author's email: michael.palmer@angstrom.uu.se

Though a large number of bioinspired and synthetic tissue adhesives have been developed, none have been granted approval by the US food and drug administration (USFDA) for use as a calcified tissue adhesive[1-6]. In fact, a comprehensive set of preclinical evaluation criteria does not exist, as there is no existing device predicate for comparison. Herein we review recently developed, novel adhesives that have been proposed or evaluated for bonding calcified tissues, and suggest potential evaluation criteria for specific clinical applications (e.g. fracture fixation). Comparisons are made between published data on modern synthetic adhesives, biomimetic adhesives, and a newly discovered class of phosphoserine cement adhesives, which appear suitable for calcified tissue reconstruction. Finally, the current preclinical standards (ISO) (e.g. lap shear testing) will be compared with cutting edge experimental test methods (e.g. in vivo adhesive-augmented screw pull-out testing in femur, combined with microcomputed tomography imaging of mechanical failure), to identify whether new preclinical evaluation methods are warranted.

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Abstract **#1518**

ASSESSMENT OF IN VITRO AND IN VIVO ASSAY TO EXPLOIT THE POTENTIAL OF CELLS AND TISSUE-ENGINEERED MEDICAL PRODUCTS: ACCELERA'S EXPERIENCE.

Simona Marzorati[P][1], Alberto Ghiglieri[1], Guido Di Gallo[1], Claudio Bernardi[1]

[1] *Accelera Srl*

Corresponding author's email: simona.marzorati@accelera.org

Due to their intrinsic variability, cells and tissue-engineered medical products require tailored and flexible preclinical safety assessment. In Accelera, the assessment of toxicity, and when required, ADME properties, of different tissue engineering and regenerative medicine products, are usually carried out adopting Good Laboratory Practice (GLP) standards. Evaluation of the stability of cellular phenotypes, delivery, and treatment localization and of cell fate is usually performed combining in vitro testing via cell culture assay and in vivo assessment in appropriate animal models. Fine-tuning and a modular strategy allow to exploit the characteristic of each component of the candidate product. For example, molecular biology tools (e.g., qPCR), integrate microscopy (e.g., Immunofluorescence), ELISA, and cytofluorimetric analysis are combined to allow the monitoring of the biodistribution of therapeutic cells or cell products. Tissue absorption, distribution, metabolism, and excretion of cellular and non-cellular materials are also exploited using autoradioluminography techniques and HPLC-MS/MS approach. Biomaterials must fulfill some requirements to be safely implanted into the human body, for this reason, when is required, radiolabeling techniques are used to improve research for regenerative medicine and prosthetic devices. With the aim to apply the 3 R principle, that promotes replacement, refinement, and reduction of animal experiments, thanks to numerous collaborations, promising new in vitro tools (e.g., organ-on-chip) are eventually explored to compare data obtained with animal models, such as the absence of toxic events (e.g., by assessing cardiovascular or liver toxicity).

Abstract **#1519**

HYALURONIC ACID: NANOCELLULOSE COMPOSITE BIOINKS FOR 3D BIOPRINTING FACIAL CARTILAGE

Thomas Jovic$_P$[1,2], Zita Jessop[1,2], Bethan Morgan[3], Karl Hawkins[3], Shareen Doak[3], Iain Whitaker[1,2]

[1] *Reconstructive Surgery & Regenerative Medicine Research Group, Swansea University, UK*

[2] *Welsh Centre for Burns and Plastic Surgery, Morriston Hospital, Swansea, UK*

[3] *Swansea University*

Corresponding author's email: thjovic@doctors.org.uk

Introduction: 3D bioprinting technology holds exciting promise for reconstructive surgery. Our previous work has shown Nanocellulose (NC) bioinks have promising biological and mechanical properties for 3D bioprinting. The aim of this project was to combine nanocellulose with hyaluronic acid for 3D bioprinting cartilage to further optimise its biomechanical strength and chondrogenicty with a view to 3D bioprint cartilage.

Methods: Hyaluronic acid (HA) powder was gelated to make a 30mg/ml hydrogel. NC and HA were blended to make composite NC-HA bioinks ranging from 100% HA to 20% HA, with NC-alginate as a control. Human nasoseptal chondrocytes were cultured in the biomaterial, crosslinked with 5µM hydrogen peroxide, for 21 days. Chondrogenicity was determined with PCR and histology. Printability was assessed using an AR-G2 Controlled Stress Rheometer. Cell viability and proliferation within the biomaterial was demonstrated with Live-Dead and AlamarBlue assays.

Results: Instant crosslinking of NCHA was achievable at concentrations of 5µM hydrogen peroxide with no detriment to cell survival. NCHA was noted to be up to 10-fold more chondrogenic ($p<0.0001$) than NC-Alginate and HA alone ($p<0.0001$). Histologically, NCHA demonstrated more intensely stained extracellular matrix than NC-Alginate and HA. Of the NCHA mixtures, 20%HA -80%NC ($p=0.0001$) was the most chondrogenic blend. Rheologically, all hydrogels demonstrated appropriate mechanical properties for 3D printing. Cell viability and proliferation was sustained over 21 days.

Conclusions: NCHA bioinks demonstrate superior chondrogenicity, favourable mechanical properties and excellent biocompatibility for bioprinting cartilage for reconstructive surgery. We explore the impact of these findings and suggest the potential long-term clinical impact.

Abstract **#1520**

THE EFFECTS OF CANINE MESENCHYMAL STEM CELL CONDITIONED MEDIUM ON ORGANOTYPIC SPINAL CORD SLICE CULTURES

Chelsea Wood$_P$[1], Peter Myint[2], John Innes[2], Esri Juarez[3], Francesco Ferrini[3], Adalberto Merighi[3], Eustace Johnson[4]

[1] *Biological Sciences, University of Chester, UK*

[2] *Veterinary Tissue Bank Ltd, Chirk, UK*

[3] *Dept. of Veterinary Sciences, University of Turin, Italy*

[4] *Chester Medical School, University of Chester, UK*

Corresponding author's email: chelsea-wood@hotmail.com

Spinal cord slice (SCS) cultures enable physiological study of complex CNS microenvironments and have been used to model neuroinflammatory responses associated with nociception. Mesenchymal stem cells (MSCs) are thought to reduce neuroinflammation and pain signalling due to their paracrine activity within the injured spinal cord. Here, explants of mouse SCSs were cultured in canine MSC conditioned medium (cMSC CM) versus control medium. There were significantly increased levels of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes, reduced levels of βIII tubulin immunopositive nerve fibres and increased calcium transients in the dorsal horn of SCSs in MSC CM versus control medium. The up-regulation in GFAP in astrocytic cells was confirmed by Western blotting of cell extracts from cMSC CM treated U-87MG glioblastoma cells (versus control medium), which is indicative of reactive astrocyte hypertrophy. These results contrast with previous studies of MSC transplants after spinal cord injury (SCI), where decreased astrocytic reactivity and increased axonal growth were reported. Hence, this study shows: i) that different experimental model systems elicit important differences in response to MSC activity; ii) suggesting that wound responses seen within the CNS are complex, and; iii) that MSC-secreted factors may reduce inflammatory responses after SCI via their effects on infiltrating inflammatory cells, rather than directly on cells within the spinal cord itself.

Abstract **#1521**

DEVELOPMENT OF ANISOTROPIC GRADIENT MINERALISED HYDROGEL MATRICES FOR OSTEOCHONDRAL TISSUE REGENERATION

Daniel David Whitep[1,2], Neil Henderson Thomson[3,2], Georg Alexander Feichtinger[2]

[1] *EPSRC Centre for Doctoral Training in Tissue Engineering and Regenerative Medicine, School of Mechanical Engineering, University of Leeds, LS2 9JT, UK*

[2] *Division of Oral Biology, School of Dentistry, University of Leeds, LS2 9JT, UK*

[3] *Molecular and Nanoscale Physics Group, School of Physics and Astronomy, University of Leeds LS2 9JT, UK*

Corresponding author's email: el11dw@leeds.ac.uk

Due to the limited regenerative capacity of cartilage, patients that develop severe osteochondral (OC) defects require surgical intervention to restore the natural biomechanics of the joint. However, current graft treatments rely on donor tissue availability and regenerative therapies such as autologous chondrocyte implantation (ACI) and matrix-assisted ACI (MACI) require costly in-vitro cell expansions. Gene-activated matrices (GAMs) are a promising, graft-free and acellular alternative to current treatments for OC defects that use spatially controlled vectors encapsulating gene-encoding therapeutic payloads to instruct stem cell differentiation. Recently, a novel electrophoretic platform technology has been developed to produce anisotropic gradient mineralised hydrogels as GAMs for in-situ OC defect regeneration at low cost. This approach spatially controls coprecipitation of plasmid-DNA- (pDNA) loaded calcium phosphate nanoparticles (pDNA-CaP-NPs) and pDNA-loaded magnesium phosphate nanoparticles (pDNA-MgP-NPs) as osteogenic and chondrogenic layers respectively. The GAM therefore spatially controls nucleic acid drug delivery, cell transfection and biomineralisation of the gel matrix in-vivo. The bioactivity and pDNA release kinetics of monolayered and anisotropic bi-layered matrices in simulated body fluid were measured using appropriate assays and SEM imaging. Osteoconductive properties were demonstrated in the pDNA-CaP-NP layers of monolayer and bi-layer matrices due to Ca2+ and PO43- ion absorption and hydroxyapatite- (HAp) like particle precipitation. Meanwhile, pDNA-MgP-NP layers demonstrated a comparatively reduced mineralisation. The anisotropic HAp-like mineral precipitation in bi-layer matrices indicates their potential for OC tissue regeneration. Further development of the novel electrophoretic platform will allow manipulation of nanoparticle nucleation and growth mechanisms to modulate particle sizes for optimal in-vitro transfection efficiencies.

Keywords

Osteochondral; Hydrogel; Electrophoresis

Abstract **#1522**

LOW-COST 3D BIOPRINTER WITH ADAPTER FOR MULTI-MATERIAL EXTRUSION

Emerson G Moretto[P][1], Leonardo O.P. Longo[1], Fernanda C Bombaldi de Souza[1], Renata F Bombaldi de Souza[1], Tácia T.A. Liguori[1], Gabriel R Liguori[1]

[1] *TissueLabs SAGL*

Corresponding author's email: gabriel@tissuelabs.com

INTRODUCTION: The use of 3D bioprinters with multiple printheads has been proposed for the fabrication of complex tissues. The use of multiple extrusion heads requires the constant switch among the different extrusion heads, reducing print speed and efficiency and ultimately leading to increased stress for the cells. Some alternatives for this problem are the use of microfluidic systems and systems composed of multiple reservoirs connected to an extrusion unit by a tubing system. This approach, however, can either lead to material loss or restrict the use of hydrogels with high viscosity. A different approach to allow the 3D bioprinting of multiple biological materials is desirable. METHODS: We developed a 3D bioprinting system containing a syringe adapter designed to be attached to its extrusion unit. This adapter allows the extrusion of two (or more) materials, each of them flowing from a different syringe. This syringe adapter can be designed in different ways. RESULTS: Three distinct designs were proposed and carried out. The first, to extrude two or more materials, flowing from two or more syringes, as a parallel multi material flow. The second, to extrude two or more materials, flowing from two or more syringes, as an homogeneous, mixed, single material flow. The third, to extrude two or more materials, flowing from two or more syringes, as a coaxial multi material flow. We successfully 3D bioprinted tissue constructs using these different approaches. CONCLUSION: Low-cost multi-material extrusion 3D bioprinting systems can be achieved with the use of customized adapters.

Keywords

Low-cost; Multi-material; Syringe adapter

Abstract **#1524**

BLOCKAGE OF BMP SIGNALLING PATHWAY COUNTERACT HYPERTROPHY IN AN IN VITRO HUMAN OSTEOARTHRITIC MICRO-CARTILAGE MODEL

Shikha Chawla[P][1,2], Majoska H. M. Berkelaar[2], Boris Dasen[2], Sourabh Ghosh[1], Ivan Martin[2], Andrea Barbero[2], Paola Occhetta[2,3]

[1] *Indian Institute of Technology Delhi*

[2] *Department of Biomedicine, University Hospital Basel, University of Basel*

[3] *Department of Electronics, Information and Bioengineering, Politecnico di Milano*

Corresponding author's email: chawla.shikha16@gmail.com

The role of Bone Morphogenetic Protein (BMP) signalling in triggering hypertrophy during embryonic cartilage development has been demonstrated. BMP has been also recently postulated to contribute in the onset of cartilage hypertrophy during osteoarthritis (OA) pathogenesis. Our recent findings demonstrated that selective inhibition of BMP signalling contributes in counteracting hypertrophic differentiation of adult human mesenchymal progenitors (1). Motivated by this finding, we hypothesized that selective inhibition of BMP signalling by targeting ALK2 and ALK3 with LDN-193189, a commercially available BMP type I receptor kinase, would also revert hypertrophic features in OA cartilage. To test this hypothesis, we first developed a 3D in vitro OA micro-cartilage model using minimally expanded OA chondrocytes cultured in poly-2-hydroxyethyl methacrylate coated 96-well plate (2). This 3D in vitro OA micro-cartilage model reproducibly captured OA-like hypertrophic features, as demonstrated by upregulation of hypertrophy-related genes (e.g. COL10A1, MMP13 and IHH) and presence of clustered cells positive for Collagen type X. Inhibition of BMP signalling pathway by means of LDN-193189 then resulted in a reduction of such OA hypertrophic features (reduction of Collagen type X positive cell clusters, reduced expression of COL10A1,MMP13 and IHH), while maintaining the capacity of the cells to produce cartilage specific proteins. Our findings open potential pharmacological strategies to counteract cartilage hypertrophy in OA and support the broader perspective that key signalling pathways comprehended from developmental processes can guide the understanding, and possibly the counteraction, of adult pathological traits.

Keywords

BMP signalling; Hypertrophy; Osteoarthritis

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Abstract **#1525**

THE EFFECT OF LOW-ENERGY EXTRACORPOREAL SHOCKWAVE TREATMENT (ESWT) ON THE FUNCTIONAL, MORPHOLOGICAL AND MOLECULAR LEVEL IN SUB-ACUTE AND CHRONIC PHASES OF TRAUMATIC SPINAL CORD INJURY

Mohamed Ashmwep[1]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in AUVA Research Center, Vienna, Austria*

Corresponding author's email: alexander.ruehrnoessl@gmail.com

Extracorporeal shockwave therapy has proven to be effective for treating various pathologies of soft tissues, bone and the peripheral nervous system.

We investigated effects of ESWT in sub-acute (2 weeks after injury) and chronic (5 weeks after injury) settings in a rat contusion model at the 11th thoracic vertebra, at clinically relevant time points regarding secondary spinal damage. Functional outcome was assessed using BBB-Score, Ladder rung walking test and Catwalk®-analysis at different time points. Lugol-stained spinal cords accessed by µCT imaging provided insights into morphological changes after ESWT. Numerous systemic microRNAs were screened to explore therapeutic effects and underlying mechanisms of injury.

In Open Field-testing animals in both treatment settings (n=12 and n=15) showed significant improvement regarding their functional outcome in contrast to untreated rats (n=24) after SCI. However, Catwalk®-analysis did not show statistical differences between therapy and control groups in initial experiments. Currently the range of methods has been extended by ladder rung walking test and retrograde labelling, however data obtained is still being analysed.

Development of an automated computational algorithm to measure parameters of injured spinal cord, such as remaining spinal cord at lesion, revealed significant correlation between higher BBB scores at the end of observation time and a greater rate of spared white matter compared to controls. Finally, we were able to identify miRNAs (e.g.miR-375) showing significant treatment effects after ESWT.

We provide evidence for ESWT efficacy in treatment of subacute and chronic spinal cord injuries, and allow further insights into secondary mechanisms of damage after SCI.

Keywords

Shock Wave; Spinal Cord Injury

Abstract **#1527**

THE TEMPORARY APPROACH IN THE MANAGEMENT EPIDERMOLYSIS BULLOSA RELATED SQUAMOUS CELL CARCINOMA: IS IT EFFECTIVE TO USE MATRIDERM(DERMAL SUBSTITUTE) AND SUPRATHEL(DL-LACTIDE ACID POLYMER) ?

Mehmet Bozkurt[P][1], Burak Ergün Tatar[1], Percin Karakol[1], Can Uslu[1], Melihcan Sezgic[1]

[1] *Bagcılar Training and Research Hospital Department of Plastic Surgery*

Corresponding author's email: drmbozkurt@yahoo.com

INTRODUCTION:

Epidermolysis bullosa (EB) is a genetic disease. Skin cancer may develop in EB patients due to skin sensitivity. As a surgical treatment, it is treated with an appropriate reconstruction method after excision.

In this case, we present our patient whose temporary treatment was performed using Suprathel(DL-lactide acid polymer) and dermal substitute Matriderm in EB related SCC(Squamous Cell Cancer) in the dorsum of the right hand and left foot.

CASE:

A 26-year-old EB male patient presented with skin lesions on the dorsum of his right hand and left foot. The biopsy results are SCC. After oncology and orthopedic consultations, tumors in the foot and hand were excised under general anesthesia in accordance with the clean surgical margin. A partial-thickness skin graft was taken from the right thigh. Suprathel dressing was applied over the donor area with cell-spray also Matriderm and graft were inset on the foot and hand. It was covered with dressing.No complications were seen in the donor site and grafts in postoperative period.

CONCLUSION:

Skin lesions in EB increase the possibility of infection and, therefore, the possibility of mortality. The incidence of skin cancer increases in long-term skin lesions. In our patient, the suprathel used to prevent a second defect in the graft donor area in SCC treatment, and the matriderm used for the inset of the graft provided a suitable wound healing environment. Matriderm had a positive effect in terms of the use of suprathel and graft inset as temporary wound care in patients with EB.

Keywords

Epidermolysis bullosa; Squamous Cell Cancer; Dermal Substitutes

Abstract **#1529**

INDUCTION OF TENOGENIC DIFFERENTIATION IN MESENCHYMAL STEM CELLS VIA CULTURE ON TENOCYTE-IMPRINTED SUBSTRATE

Seyed Mohammad Amin Haramshahi[P][1,2], Shahin Bonakdar[3], Mehdi Moghtadaei[4], Sara Tanbakooei[1], Sara Simorgh[2], Peiman Brouki-Milan[2], Naser Amini[2], Noorahmad Latifi[5], Ali Samadikuchaksaraei[2], Mansoureh Soleimani[2]

[1] *Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran*

[2] *Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran*

[3] *National Cell Bank, Pasteur Institute of Iran, Tehran, Iran*

[4] *Department of Orthopedic Surgery, Rasoul-e-Akram Hospital, Iran University of Medical Science, Tehran, Iran*

[5] *Department of Plastic and Reconstructive Surgery, Hazrat Fatemeh Hospital, Iran University of Medical Sciences, Tehran, Iran*

Corresponding author's email: sma.haramshahi@gmail.com

Differentiation of stem cells into mature tenocytes using biophysical signals has been interested in recent years. In this study, we used human tenocytes and sections of tendon tissue as a positive mold to make a polydimethylsiloxan-based patterned substrate for the induction of tenogenic differentiation in mesenchymal stem cells.

For making patterned sheets, PDMS were cast on fixed tenocytes and longitudinal sections of human tendon. Adipose derived mesenchymal stem cells were cultured on patterned sheets (including tenocyte imprinted PDMS; that called cell replica group and tendon imprinted PDMS that called Tissue replica group) and after 7 and 14 days the expression of tenogenic markers including Scleraxis (SCX), Tenomodulin (TNMD) and Tenascin C (TNC) were evaluated using real time RT-PCR.

On the seventh day after culture, only TNC marker in the Tissue replica group showed a significant increase compared to the control group. Although TNMD and SCX expression increased in both groups, this increasing did not reach a significance level. Also, on the fourteenth day after culture, the expression of TNMD and SCX markers showed a significant increase only in the cell replica group compared to the control group.

The results of this study showed that a proper surface topography can induce tenogenic differentiation in stem cells. Oue findings also suggest that induction of adult cell morphology in stem cells can be considered as a way to induce differentiation and can be used as a strategy for tissue engineering purposes.

Abstract **#1530**

PHYSIOLOGICALLY RELEVANT ASSESSMENT OF BIOMATERIALS IN BIOMIMETIC BIOREACTORS AIMED FOR REGENERATION OF SKELETAL TISSUES

Bojana Obradovic[1], Jasmina Stojkovska[1], Jovana Zvicer[1], Ivana Banicevic[1], Ana Medic[2,3], Sasa Novak[2,3], Djordje Veljovic[1], Vesna Miskovic Stankovic[1]

[1] *Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

[2] *Department for Nanostructured Materials, Jozef Stefan Institute, Ljubljana, Slovenia*

[3] *Jozef Stefan International Postgraduate School, Ljubljana, Slovenia*

Corresponding author's email: bojana@tmf.bg.ac.rs

Development of biomaterials aimed for biomedical utilization requires extensive physico-chemical characterization followed by in vitro and in vivo functional evaluation. This process is long and expensive imposing the need for methods for quick and reliable selection of the most promising candidates. Biomimetic bioreactors are recognized as valuable tools that can provide evaluation of biomaterial functionality under physiologically relevant conditions, biomechanical properties, and cell–biomaterial interactions. In the present study, we address all of these three aspects by utilizing two bioreactor types: perfusion and a bioreactor with dynamic compression. Specifically, we evaluated bioactivity of porous composite scaffolds based on hydrogels (gellan gum and alginate) and hydroxyapatite (HAp) precursor particles (bioactive glass and beta-tricalcium phosphate) aimed for bone tissue regeneration. Perfusion of simulated body fluid at superficial velocities in the range 100-500 µm/s relevant for osteogenesis was shown to significantly enhance HAp deposition revealing mass transfer limitations in traditional static tests. The bioreactor with dynamic compression applied in the regime relevant for articular cartilage (5 % deformation, 0.68 Hz frequency, 337.5 µm/s loading rate) was utilized to monitor biomechanical properties of scaffolds over time revealing slight composite weakening at lower mineral contents while an increase in dynamic compression modulus at pronounced HAp deposition. Finally, the same bioreactors were utilized for cytotoxicity evaluation of nanocomposite Ag/alginate hydrogels as potential antimicrobial cartilage implants as well as cultivation of osteosarcoma cell line in a bioactive osteogenic environment. These studies confirmed advantages of biomimetic bioreactors in providing consistent results corresponding to those found in vivo.

Abstract **#1531**

DEVELOPMENT OF A TISSUE REGENERATIVE SCAFFOLD PLATFORM TECHNOLOGY

Simge Bahcevanci[P][1], Hollie Hathaway[2], Victor R. de la Rosa[3], Suzan Aksakal[3], Julian Dye[1], Hua Ye[1]

[1] *University of Oxford*

[2] *ConvaTec Group PLC*

[3] *Ultroxa Polymers. Avroxa BV, Ghent, Belgium*

Corresponding author's email: simge.bahcevanci@eng.ox.ac.uk

Traumas can cause complex wounds require tissue regenerative scaffolds1. Scaffold design requires optimization of mechanical strength, biodegradability, porosity, interconnectivity, and antigenicity2. The project aims to develop biodegradable, regenerative soft tissue scaffolds for reconstructing acute and chronic wounds. A unique high internal phase macro-emulsion(HIPE) scaffold formulation was developed in our lab, providing a hierarchical microporous nanostructure scaffold based on native proteins3. Oil-in-water emulsions were prepared with protein and polymer components, cast at 37ºC fallowing crosslinking, reducing, washing, and lyophilisation steps. The resulting product provides angiogenic properties due to fibrin`s binding sites for ECs resulting high cell response4.Natural and synthetic polymers give finely distributed fibrin meshes with high porosity/interconnectivity, and controllable pore void diameter of 100-200µm, shown by SEM images. Cytotoxicity assay using the PBS extract of scaffolds revealed that the crosslinking step does not cause cytotoxicity and the viability is around 65%-73%. Mechanical analysis shows that the scaffolds have storage modulus between 0.30-0.56 MPa while young's modulus between 0.065-0.090 MPa, suggesting polymer addition provides higher young's modulus and higher elasticity. These preliminary results show that the scaffolds yield encouraging visco-elastic properties for surgical handleability and the polymer composites can provide control over the mechanical properties. These results demonstrate the potential for optimisation of mechanical properties, biological response, degradation kinetic and interior structure as the crucial design parameters. The synthetic and natural polymer/protein blends effect in HIPE scaffolds will enable the creation regenerative scaffolds with novel properties and will be an important milestone in the pursuit of a next-generation skin scaffold.

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Abstract **#1532**

TWO-PHOTON MICROPATTERNING OF 3D BONE CELL NETWORKS IN PHOTOSENSITIVE HYDROGELS

Christian Gehre[P][1], Ralph Müller[1], Xiao-Hua Qin[1]

[1] *Institute for Biomechanics, ETH Zurich*

Corresponding author's email: cgehre@ethz.ch

Crucial for the structural integrity of bone – and thus vertebrate survival – is a functional cellular network throughout the bone matrix. To form this network, mechanosensing cells called osteocytes are deeply embedded inside the bone, creating an interconnected cellular system [1]. It is recognized that the microarchitectural environment of these networks influences the osteocytes' ability to orchestrate the dynamic process of bone remodelling [2]. However, osteocyte mechanotransduction inside the complicated lacuna-canalicular-network (LCN) remains poorly understood. To overcome the inaccessible nature of dense bone in order to access osteocytes, we combined multiphoton-based laser ablation with 3D bone cell culture in photodegradable hydrogels [3, 4]. With this approach, we were able to embed human mesenchymal stem cells (hMSC) in gelatin methacryloyl (GelMA) hydrogels and perform subtractive multiphoton patterning of microchannels. Patterning in the presence of bone cells lead to the outgrowth of cellular protrusions into the artificial canaliculi, similar to osteocyte dendrites in bone. Despite the invasive process of 3D subtractive laser printing, the cells showed remarkable cell viability and could be kept under osteogenic culturing conditions for at least 14 days. To confirm the cellular phenotype, the expression of differentiation biomarkers will be investigated alongside utilization of other photodegradable materials, such as polyethylene glycol diacrylates. Taken together, these results show the promising effect of multiphoton based laser ablation of photosensitive hydrogels towards the formation of 3D osteocyte networks in vitro.

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Abstract **#1533**

3D PRINTED POLYMERIC BIORESORBABLE STENT AS MULTIFUNCTIONAL SCAFFOLDS FOR CORONARY ARTERIES

Marta Pegueroles[1,2], Victor Chausse[1], Santiago Raymond[1], Kumaran Kolandaivelu[3], Cristina Canal[1,2], Carlos Mas-Moruno[1,2], Maria-Pau Ginebra[1,2]

[1] *Biomaterials, Biomechanics and Tissue Engineering Group, Technical University of Catalonia (UPC),Spain*

[2] *Barcelona Research Center in Multiscale Science and Engineering, UPC, Spain*

[3] *Institute for Medical Engineering and Science, MIT, Cambridge, USA*

Corresponding author's email: marta.pegueroles@upc.edu

Bioresorbable stents (BRS) are designed to overcome perceived limitations of drug-eluting stents (DES) by providing temporary support to the vessel wall [1,2]. Herein, we developed 3D printed BRS by solvent-cast direct-write technique as a multifunctional scaffold able to encapsulate drugs and/or to attach peptides and/or to be radiopaque to overcome coronary problems. 3D printed stents were obtained with a modified fused deposition modelling (FDM) 3D printer (BCN 3D). The ink was a solution of poly-l-lactic acid (Purasorb) in chloroform (3.7%v/v). Inks were modified: (i) with iodine to render radiopacity (0.1-1% w/v) or (ii) with antithrombogenic drug aspirin (0.2% w/v).REDV and YIGSR peptides were synthesized by solid-phase and covalently attached to the surface using EDC/NHS. Printed stents were characterised by SEM and DSC and evaluated with expansion and compression mechanical tests. Radiopacity of iodinated stents was assessed in a µ-CT scanner. Aspirin release was characterised by spectrophotometry. Thrombogenicity was tested into a prefusion chamber with volunteer donnor's blood. PLLA biodegradable stents of 3 mm diameter were obtained with struts of 130 µm or 80 µm and different peaks. Iodinated stents manifested radiopacity at the expense of a decrease in radial strength. Aspirin-loaded stents showed an initial burst and posterior steady release of the drug. Average breaking pressure was between 15-17 atm and a good stent deployment was observed into the simulated artery. Biological tests confirmed that 3D printed stents were not cytotoxic independently of the number of peaks. Finally, functionalization enhanced HUVEC migration and did not increase thrombogenicity. Acknowledgements: RTI2018-098075-B-C21

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Abstract **#1534**

NEXT GENERATION BIOENGINEERED HUMAN MYOCARDIUM

Alain van Mil[P][1,2], Miguel Castilho[1,3,4], Nino Chirico[1,2], Madison J. Ainsworth[1,3], Inge Dokter[1,2,3], Renée G.C. Maas[1,2], Jan Willem Buikema[1,2], Jos Malda[1,3,5], Joost P.G. Sluijter[1,2]

[1] *Regenerative Medicine Center Utrecht, Circulatory Health Laboratory, University Medical Center Utrecht, Utrecht, the Netherlands*

[2] *Laboratory of Experimental Cardiology, Department of Cardiology, University Medical Center Utrecht, Utrecht, the Netherlands*

[3] *Department of Orthopaedics, University Medical Center Utrecht, Utrecht, the Netherlands*

[4] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands*

[5] *Department of Equine Sciences, Faculty of Veterinary Sciences, Utrecht University, Utrecht, the Netherlands*

Corresponding author's email: A.vanMil@umcutrecht.nl

Cardiac patches consisting of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) show beneficial effects when placed on the infarcted heart and the first human clinical trials have been approved. However, current patches do not replicate myocardial tissue, lacking 3D organization, mechanical properties, cellular maturity, and relevant thickness, and thereby fail to provide real contractile support to the failing heart.

Previously, we have shown that melt electrowritten (MEW) hexagonal fiber scaffolds can be used to generate contractile cardiac patches that mimic native mechanical properties, thereby inducing iPSC-CM maturation and tissue organization.[1] Although a huge step forward, these constructs do not yet fully replicate myocardial cellular and ECM composition and organization, and myocardial 3D fiber alignment.

To tackle these hurdles, we have investigated the incorporation of additional cardiac cell types like iPSC-derived cardiac fibroblasts (cFBs) and endothelial cells (ECs), the use of various myocardial and EC-optimized bioinks for extrusion-based bioprinting to allow for strategic cell-type arrangement, and introducing 3D myocardial fiber-angle orientation by stacking hexagonal MEW scaffolds.

We found that the addition of cFBs, the optimization of hydrogel/ECM composition and stiffness (Collagen-GelMA), and increasing thickness (1cm) and fiber organization by strategically stacking hexagonal meshes, led to the formation of a thick synchronously contracting myocardial tissue-like construct. Our constructs showed a multi-layered 3D fiber organization, with cells aligning with the hexagonal microarchitectures and an increase in maturation.

Taken together, we have developed a next-generation bioengineered myocardium with a more native-like muscle structure and the potential to provide real functional support to the injured heart.

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Abstract **#1535**

GIOTTO CEMENT: A NOVEL INJECTABLE, RESORBABLE AND PRO-OSTEOGENIC DEVICE TO TREAT OSTEOPOROTIC VERTEBRAL COMPRESSION FRACTURES

Federica Banche-Niclot$_P$[1,2], Ilaria Corvaglia[1], Elena Boggio[3], Casimiro Luca Gigliotti[3], Umberto Dianzani[3], Nicholas Dunne[4], Antonio Manca[5], Sonia Fiorilli[1], Chiara Vitale-Brovarone[1]

[1] *Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy*

[2] *Department of Surgical Science, Università degli Studi di Torino, Torino, Italy*

[3] *NOVAICOS s.r.l.s., Novara, Italy*

[4] *Centre for Medical Engineering Research, School of Mechanical and Manufacturing Engineering, Dublin City University, Dublin, Ireland*

[5] *Department of Radiology, Candiolo Cancer Institute-FPO-IRCCS, Candiolo, Turin, Italy*

Corresponding author's email: federica.banche@polito.it

Approximately 200 million people worldwide are suffering from osteoporosis[1],a metabolic bone disease characterized by low bone density and deterioration of bone architecture that increases the risk of fracture[2].Vertebral compression fractures are extremely frequent in osteoporotic patients, resulting in back pain and immobility. In the frame of the H2020 GIOTTO project, an innovative composite cement was developed to stabilize these fractures and to stimulate bone regeneration. The cement was prepared by combining a mixture of inorganic phases in dry powder form with an aqueous phase to obtain a paste-like material, directly injectable into the fractured vertebral bodies. The powder component consisted of α-calcium sulphate hemihydrate as resorbable matrix, mixed with strontium and zirconium containing mesoporous bioactive glasses to impart pro-osteogenic abilities and radiopacity, respectively. The effects of liquid-to-powder ratio on cement's injectability and setting time were investigated. Furthermore, to confer anti-osteoclastogenic properties to the final device, ICOS-Fc molecule able to inhibit the osteoclast activity was incorporated into the final formulation.The injectability was tested in a human vertebral body model obtained from human tomography images. The setting time of the developed paste and the related rheological behavior along setting were evaluated. The imparted radiopacity and the presence of residual porosity, detrimental to the cement mechanical properties, were examined using micro-computed tomography. In vitro release of Sr2+ions and ICOS-Fc, as well as the degradation kinetics of the cement were investigated under simulated physiological conditions. Static mechanical testing under compressive loading was also conducted. The GIOTTO cement overall properties were compared to commercial references.

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Abstract **#1536**

BIOACTIVE GLASSES AND THE IMMUNE SYSTEM: RECENT FINDINGS AND FUTURE PERSPECTIVES

Aldo Boccaccini$_P$[1], Katharina Schuhladen[1], Alexander Steinkasserer[1], Elisabeth Zinser[1]

[1] *University of Erlangen-Nuremberg*

Corresponding author's email: aldo.boccaccini@ww.uni-erlangen.de

Although bioactive glasses (BGs) are being increasingly explored for various applications in tissue engineering and regeneration [1], the effects of ions released by BGs on the immune system, surprisingly, have not been investigated in detail so far. In this presentation current understanding about the effect of BG dissolution products on cell biology markers characterising the immune system response will be summarized. The results of recent investigations involving silicate and borate bioactive glasses with tailored delivery of biologically active ions will be discussed. Dendritic cells, being the most potent antigen-presenting cells of the immune system, were investigated in their interaction with different concentrations of ionic dissolution products from ion-doped borate BGs [2]. The borate BGs were based on the well-known silicate 13-93 composition and additionally doped with copper and/or zinc. A dose-dependent effect on the viability and expression of surface markers of dendritic cells was found by cultivating dendritic cells in the presence of different concentrations of BG dissolution products. Moreover, by co-cultivating dendritic cells and T-cells, a composition and dose-depending effect of the investigated BGs was found on the capability of dendritic cells to induce T-cell proliferation. The effect of the relative ion concentration was specially considered taking into account different degradation rates of BGs. Therefore, the results of this study showed the immune modulating effects of BGs, and open an important area of research anticipating that the response of the immune system can be greatly affected by the type and concentration of metallic ions released from BGs.

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Abstract **#1537**

DYNAMIC OXYGEN PRE-CONDITIONING OF MESENCHYMAL STROMAL CELLS TO ENHANCE THERAPEUTIC ANGIOGENESIS FOR REGENERATIVE MEDICINE PURPOSES

Jasmine Ho[P][1], Martin Birchall[1], Mark Lowdell[1], Umber Cheema[1]

[1] *University College London*

Corresponding author's email: jasmine.ho@ucl.ac.uk

INTRODUCTION:

Hypoxia is a key factor for the up-regulation of angiogenic factors in mesenchymal stromal cells (MSCs).1 Our aim is to determine the exact in vitro oxygenation conditions through dynamic pre-conditioning in 2D and 3D settings, which can help further potentiate the angiogenic pathway for therapeutic in vivo applications.

METHODS:

MSCs were pre-conditioned in normoxia (21% O2) and physiological hypoxia (1% and 5% O2) and subsequently seeded into Type I collagen hydrogels. These cell-loaded 3D hydrogels were subjected to three different oxygen conditions (1%, 5% and 21%). Media from different time points were collected and analysed for VEGF levels and RNA from cells were extracted for qPCR of HIF1a and stemness markers. Viability and proliferative capacity were also determined.

RESULTS:

Cells pre-conditioned in 2D in physiological hypoxia (5% O2) prior to being seeded did not exhibit increased VEGF production when compared to cells pre-conditioned in normoxic (21% O2) conditions. Cell viability between cells grown in 21% and 5% were not significantly different, but proliferative ability decreased when cells were cultured in 1% O2. Normoxic pre-conditioned cells produced more angiogenic factors and HIF1a gene upregulation when subsequently placed in 3D hypoxic conditions (5% and 1%), than if they were pre-conditioned in hypoxia and then cultured in 3D hypoxia. Hypoxia enabled maintenance of stemness markers.

CONCLUSION:

Our results offer an insight into cellular behaviour of MSCs within their 2D and 3D settings in dynamically changing oxygen environments. This could help guide pre-conditioning of cells in the pre-clinical stages for therapeutic angiogenesis purposes.

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Abstract **#1538**

VISIBLE LIGHT CROSSLINKED NITRIC OXIDE RELEASING HYDROGEL FOR RAPID WOUND HEALING

Anwarul Hasan$_P$[1,2], Alap Zahid[1,2], Robin Augustine[1,2], Raza ur Rehman Syed[2,1], Rashid Ahmed[2,1]

[1] *Department of Mechanical and Industrial Engineering, College of Engineering, Qatar University, Doha 2713, Qatar*

[2] *Biomedical Research Center, Qatar University, Doha, 2713, Qatar*

Corresponding author's email: alapzahid@gmail.com

Nitric oxide (NO) is a useful endogenous agent for its potential to promote angiogenic activity that is one of the essential requirements for the healing of wounds. The beneficial effect of exogenous NO for facilitating faster healing of acute and chronic wounds have been demonstrated by earlier works [1-3]. There are several biopolymers that can be used in drug delivery applications. One of them is Gelatin methacryloyl (GelMA) hydrogels which is highly favorable to physiological ECM conditions leads to rapid wound healing. GelMA was crosslinked using a cell-friendly ruthenium based crosslinker.In this context, we developed a GelMA based hydrogel patches containing a nitric oxide donor (SNAP) at an optimum level to promote cell proliferation, migration, and viability.

The NO-releasing GelMA hydrogels showed a prolonged and sustained release for a time of 120 hours. The results showed a greater number of live cells in NO-releasing GelMA hydrogel as compared to control and blank GelMA hydrogel. A higher level of NO help in promoting enhanced cell remodeling and proliferation as observed in earlier studies [1].

Our results invariably demonstrated that the incorporation of NO loaded GelMA hydrogel promoted cell proliferation, migration, and viability. The presence of NO at an optimum level promoted angiogenesis and wound healing. Initial results are highly promising towards the development of a clinically relevant GelMA based wound healing patch for the treatment of chronic diabetic wounds.

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Abstract **#1539**

PROTEOMICS ANALYSIS OF HUMAN INTESTINAL ORGANOIDS DURING HYPOXIA AND REOXYGENATION AS A MODEL TO STUDY ISCHEMIA-REPERFUSION INJURY

Anna M Kip[P][1], Zita Soons[1], Ronny Mohren[2], Annet AM Duivenvoorden[1], Berta Cillero-Pastor[2], Cornelis HC Dejong[1], Ron MA Heeren[2], Steven WM. Olde Damink[1], Kaatje Lenaerts[1]

[1] Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands

[2] Maastricht Multimodal Molecular Imaging Institute (M4I), Maastricht University, Maastricht, The Netherlands

Corresponding author's email: m.kip@maastrichtuniversity.nl

Intestinal ischemia-reperfusion (IR) is a potentially life-threatening condition. Although previous animal and human in vivo studies have provided important insights in IR pathophysiology, the inability to translate findings to effective therapies contributes to its continued high mortality rates. A physiologically relevant in vitro model is crucial to thoroughly investigate mechanisms of IR-induced epithelial injury and test potential therapies. In this study we demonstrate the use of human small intestinal organoids to model IR injury by exposing organoids to hypoxia and reoxygenation (HR). A mass-spectrometry-based proteomics approach was applied to characterize organoid differentiation and decipher protein dynamics and molecular mechanisms of IR injury in crypt-like and villus-like human intestinal organoids.

We showed successful separation of organoids exhibiting a crypt-like proliferative phenotype, and organoids exhibiting a villus-like phenotype, enriched for enterocytes and goblet cells. Functional enrichment analysis of significantly changing proteins during HR revealed enrichment of processes related to mitochondrial metabolism and organization, lipid metabolism, and immune response in both organoid phenotypes. Changes in protein metabolism, as well as mitophagy and protection against oxidative stress were more pronounced in crypt-like organoids, whereas cellular stress and cell death associated protein changes were more pronounced in villus-like organoids.

Our results demonstrate that the human intestinal organoid model allows separate investigation of the crypt- and villus response to HR, and findings recapitulate in vivo properties of the IR response. This model can be used for mechanistic studies to better understand IR pathophysiology as well as to test therapeutics to prevent IR injury and promote regeneration.

Abstract **#1540**

DEEP LEARNING AUGMENTED DIGITAL PATHOLOGY – TOWARDS COMPUTATIONAL PRECISION PATHOLOGY DIAGNOSTICS.

Peter Boor[P][1]

[1] *RWTH Aachen University*

Corresponding author's email: pboor@ukaachen.de

Pathology is essential for the diagnosis of plethora of diseases and a major read-out of experimental pre-clinical and clinical studies. The advancement in digital pathology , i.e. digitalization of histological slides, and the application of artificial intelligence, especially deep learning, has opened new research perspectives that have the potential to transform diagnostic pathology into quantitative “computational” pathology. This requires specific infrastructure, multidiscinplinary expertise and relevant large datasets. Proof-of-concept studies, including our own, open new frontiers in mining digital histology datasets. Given that pathology also provides the basis for development of in vitro models, the transformation of pathology towards a quantitative and reproducible precision medicine tool could also facilitate validation of such models.

Abstract **#1541**

THE COOPERATION OF OSTEOGENESIS AND ANGIOGENESIS IN ADSC-DERIVED SPHEROIDS

Daria Revokatova[P][1,2], Anastasia Gorkun[1], Irina Zurina[1], Denis Nikishin[3], Nastasia Kosheleva[1,2], Ekaterina Ustinova[1], Tamara Kolokoltsova[1], Irina Saburina[1]

[1] *FSBSI Institute of General Pathology and Pathophysiology, Moscow, Russia*

[2] *Lomonosov Moscow State University, Faculty of Biology, Moscow, Russia*

[3] *Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Russia*

Corresponding author's email: revokatova.d@gmail.com

To provide better regeneration in vivo bone bioengineering techniques are aimed on pre-vascularization of bioequivalents. However, the cooperation of molecular pathways regulating osteogenesis and angiogenesis in vitro is still poorly understood, with insufficient vascularization in artificial bone tissue still being one of the challenges in tissue engineering. Thus, this study set out to investigate the sequence of activation of angiogenic and osteogenic factors in human adipose-derived stromal cells (ADSCs) 3D culture during cell differentiation, induced simultaneously in two directions. Four groups of spheroids (intact spheroids, spheroids with osteogenic induction, endothelial induction, and double induction) were analyzed by qPCR and immunocytochemical staining. Complex analysis of expression of key factors in two experimental repeats indicated that ADSC spheroids were capable of spontaneous differentiation in both directions. The addition of exogenous inducing agents resulted in the modification of this process with the angiogenesis-related genes further promoting osteogenesis. The obtained experimental data suggested that the predominance of one differentiation direction and the maturity of the resulting types of cells were possibly dependent on the ADSCs subpopulations (CD90/CD105/CD73high and CD90/CD105/CD73low) randomly distributed in spheroids. The current study showed that ADSC spheroids were capable of spontaneous differentiation, while double-induction stimulated osteogenesis, also providing angiogenesis. These findings can contribute to a better understanding of the cross-talks of differentiation processes and open new approaches to the generation of vascularized bone tissue bioequivalents.

This work was supported by the Russian Federation President Grant MK-3776.2019.4 and the Special Federal Programme of the Russian Federation Government, Research Project No. 0520-2019-0026.

Abstract **#1542**

TISSUE TO ORGAN LEVEL EVALUATION OF HEART VALVE SCAFFOLD PERFORMANCE UNDER DYNAMIC LOADING CONDITIONS

Drake Pedersen[P1], Francesco Madonia[2], Antonio D'Amore[3,4], William Wagner[1,5,4]

[1] *Department of Bioengineering, University of Pittsburgh*

[2] *Università degli Studi di Palermo*

[3] *Fondazione RiMED*

[4] *Department of Surgery, University of Pittsburgh*

[5] *Department of Chemical Engineering, University of Pittsburgh*

Corresponding author's email: ddp17@pitt.edu

Introduction: Regenerative therapies targeting cardiac valves are a vibrant field of research. In attempts to stimulate tissue growth from tissue engineered heart valve (TEHV) scaffolds, taking cues from the native environment of heart valve tissue is vital. The relationship between mechanical loading and cellular activity is well-documented and represents a source of biological inspiration for TEHV design. This effort sought to analyze valve performance from tissue to organ-level scale under dynamic conditions.

Methods: TEHVs electrospun from blends of elastomeric poly(carbonate urethane)urea (PCUU) and polycaprolactone (PCL) meant to vary mechanical stiffness were placed into a mock circulatory system. 3D positions of markers placed on valve leaflets were acquired to map principal strains. In addition, the geometric orifice area (GOA) of the valves and pressure gradient during peak systole was determined.

Results: All valves were able to reproduce pressure and flow waveforms that are physiologically normal for the aortic valve. Valves were observed to coapt during diastole with no visual signs of regurgitation. Average principal strains of PCUU were 15.8% during peak systole while GOA = 1.4 cm2. By contrast, principal strains of stiffer 75%PCUU/25%PCL averaged 3.2%, with GOA = 1.1 cm2.

Conclusion: Strain magnitude was greatly reduced in the stiffer PCUU/PCL blend, demonstrating the effect of polymer choice on valve function. However, the reduction of strain also reduced movement of the valve and GOA. While TEHVs are currently made from a variety of materials, the stiffness of typical tissue engineering polymers may make them unsuitable for this application.

Abstract **#1543**

3D CONE-SHAPED SCAFFOLD ASSOCIATED WITH A BLOOD CLOT FACILITATE THE CELL HOMING FOR DENTAL PULP REGENERATION

Lisa Terranova$_P$[1,2], Guy Schlatter[1], Anne Hébraud[1], Aurélien Louvrier[3], Gwenaël Rolin[3], Christophe Meyer[3], Florent Meyer[2]

[1] *Institut de Chimie et Procédés pour l'Energie, l'Environnement et la Santé ICPEES UMR 7515, CNRS, Université de Strasbourg, Strasbourg, France*

[2] *Inserm UMR 1121, Biomaterials and Bioengineering, Université de Strasbourg, Strasbourg, France*

[3] *Inserm, EFS BFC, UMR1098, Interactions Hôte-Greffon-Tumeur/Ingénierie Cellulaire et Génique, Université Bourgogne Franche-Comté, F-25000 Besançon, France*

Corresponding author's email: lisaterranova@outlook.com

The purpose of this study was to develop a biomaterial in the form of a cone for endodontic regeneration based on cell homing (1). The cone could be inserted inside the main root canal of a tooth after preparation. It should support the formation of a stable blood clot which will act as a natural source of mesenchymal stem cells and growth factors (2).
Porous membranes made of polylactic acid and polycaprolactone were fabricated using electrospinning combined with electrospraying, and functionalized with tannic acid (TA) which bring antibacterial properties. Membrane sheets were rolled as a cone and stabilized with a gelatin coating. The biocompatibility was assessed by in vitro tests on dental pulp stem cells (DPSCs). The cones were inserted into newly designed micro-bioreactors mimicking the root canal space to study cell migration. Blood clot formation was evaluated by immersing the cone in blood from human donors.
The biocompatibility of the membranes has been demonstrated, DPSC could adhere, proliferate, differentiate in odontoblasts and colonize all the cone. TA, released in solution, showed antibacterial activity against bacteria as E.Coli. Blood coagulation with a dense fibrin network and platelet adhesion around the cone were observed by scanning electron microscopy.
Activation of platelets allowed the liberation of growth factors such as VEGF and PDGF which were detected by Elisa tests. The cones showed interesting capacities to promote cell differentiation, migration and to induce the formation of a stable blood clot. These results are promising for the regeneration of dental pulp by cell homing.

Keywords

Dental pulp regeneration; Blood clot; Cell homing

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Abstract **#1547**

TOWARDS ALVEOLAR PROGENITOR DIFFERENTIATION USING LUNG EXTRACELLULAR MATRICES-DERIVED HYDROGEL

Afshin Noori[P][1,2], Yaser Tahamtani[2,3], Sarah Rajabi[2], Fateme Ganji[4], Sirous Sadeghian Chaleshtori[5,6]

[1] *Graduated of Veterinary Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2] *Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran*

[3] *Interdisciplinary Department for Diabetes, Obesity and Metabolism, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran*

[4] *Department of Tissue engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine Iran University of Medical Sciences, Tehran, Iran*

[5] *Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[6] *Institute of Biomedical Research, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

Corresponding author's email: afshin_noori92@yahoo.com

Efficient differentiation of pluripotent cells to alveolar epithelial cells and achieving to functional mature cells was usually poor. Interaction between cells and the extracellular matrix (ECM) provides a dynamic environment and mediates cellular responses during the development and tissue repair. ECM-derived hydrogel contains proteins and glycosaminoglycan components of the native matrix and provide familiar binding site for cell attachment and important cues to guide cell differentiation.

To explore the possibility of generation of mature alveolar epithelial cells in vitro, we compared the efficiency of differentiation cells grown on lung ECM-derived hydrogel (Gel) with cells those cultured on fibronectin-coated plates. We found that the percentage of cell viability showed no significant differences in both groups after 7 days. While, both procedures revealed differentiation to definitive endoderm by expression of SOX17 and FOXA2 markers and lung progenitors' differentiation by emerging of NKX2.1 and P63 expressing cells. Whereas, cells grown on lung ECM-derived hydrogel showed significant upregulation of SOX9 as distinct marker of distal airways epithelial and developing alveolar cells and SPC (AT2 marker) as well as upregulation of differentiated cells markers including ciliated (FOXJ1) and secretory (MUC5A) cells. We concluded that the culture of PSCs-derived lung progenitors on hydrogel resulted in high upregulation of AT2 genes in comparison with fibronectin.

Keywords: Decellularized lung, ECM, Hydrogel, PSCs, lung progenitors, alveolar cells

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Abstract **#1548**

SKIN REGENERATION USING XENOGENEIC ACELLULAR DERMAL MATRIX FROM ANIMALS OF DIFFERENT AGES

Amir Mellati[P][1]

[1] *Department of Tissue Engineering, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences*

Corresponding author's email: amirmellati@gmail.com

Despite lots of attempts, wound treatment after deep trauma and severe burns remained a challenge so far. Among various approaches, acellular dermal matrix (ADM) from xenogeneic sources, including bovine ADMs are promising options. However, effects of the animal developmental stage on physical, biomechanical and biological properties of the ADMs as a skin substitute have not been fully addressed yet.

In this study, fetal (6 months) and cattle (12 months after birth) dermis were decellularized and the resulting ADMs were characterized in vitro and were evaluated in rat models.

Real-time RT-PCR analysis revealed that the ratio of collagen type III to collagen type I in fetal skin was 36.3 ± 2.1 % higher than cattle skins. Both fetal and cattle dermis were effectively decellularized through the selected protocol as confirmed by H&E staining and DNA quantification. Scanning electron microscopy of ADMs showed no significant morphological differences between fetal and cattle. Tensile strength and suture retention force were both beyond the acceptable limits (not less than 5 N per square mm and 5 N, respectively). Contraction of wounds covered with fetal ADMs was significantly faster than that of cattle ADMs during 14 days after implantation.

The results suggest that the animal age affects the performance of the ADMs in wound healing which can be correlated to their relative collagen type III content and consequently, fetal ADMs are more appealing candidates for skin regeneration.

Keywords

Acellular Dermal Matrix; Skin Regeneration; Different Ages

Abstract **#1549**

THE IMPACT OF SPACE IN HUMAN BRAIN ORGANOIDS

Alysson Muotri[P][1]

[1] *UC San Diego*

Corresponding author's email: muotri@ucsd.edu

Gravity is an organizing force in the development of tissues that influence many interactions during tissue growth. Microgravity has been shown to evoke changes in cell growth, differentiation, cell communication, aging, and epigenetic/gene expression alterations in a variety of cell types including brain cells. Moreover, exposure to microgravity has an impact on the cognition of mice and humans, suggesting that the consequences of this environment to brain function could be significant. Importantly, no complex human model of neurodevelopment was ever exposed to microgravity. Brain organoids, a new class of brain surrogate generated from human pluripotent stem cells (hPSCs), have gained traction as a model for studying the intricacies of the human brain by using advancements in stem cell biology to recapitulate aspects of the developing human brain in vitro. Recent observations from our lab revealed the spontaneous emergence of neural oscillations from these organoids, suggesting this reductionist model can be used to measure the molecular, cellular, and network impact of the space environment in the human brain. I will present data on how human brain organoids were affected by growing at the ISS.

Abstract **#1550**

GOLD NANOPARTICLES-BASED PLATFORMS FOR DIAGNOSIS AND TREATMENT OF MYOCARDIAL INFARCTION

Mojtaba Falahati$_P$[1], Anwarul Hasan[2], Farnoosh Attar[3]

[1] *Department of Nanotechnology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*

[2] *Department of Mechanical and Industrial Engineering, College of Engineering, Qatar University, Doha 2713, Qatar*

[3] *Department of Food Toxicology, Research Center of Food Technology and Agricultural Products, Standard Research Institute (SRI), Karaj, Iran*

Corresponding author's email: mojtaba.falahati@alumni.ut.ac.ir

In recent years, an increasing rate of mortality due to myocardial infarction (MI), has led to the development of nanomaterials especially gold nanoparticles (AuNPs) as promising nanomaterials for diagnosis and treatment of MI. These promising NPs have been used to develop different nanobiosensors, mainly optical sensors for early detection of biomarkers as well as biomimetic/bioinspired platforms for cardiac tissue engineering (CTE). Therefore, in this review, we presented an overview on the potential application of AuNPs as optical (surface plasmon resonance, colorimetric, fluorescence, and chemiluminescence) nanobiosensors for early diagnosis and prognosis of MI. On the other hand, we discussed the potential application of AuNPs either alone or with other NPs/polymers as promising three-dimensional (3D) scaffolds to regulate the micro-environment and mimic the morphological and electrical features of cardiac cells for potential application in CTE. Furthermore, we presented the challenges and ongoing efforts associated with the application of AuNPs in the diagnosis and treatment of MI. In conclusion, this review may provide outstanding information regarding the development of AuNP technology as a new generation platform for current MI treatment approaches.

Abstract **#1551**

IN VIVO BIOCOMPATIBILITY OF AGAROSE BIOMATERIALS USED IN TISSUE ENGINEERING

Fernando Campos[P][1], Jesús Chato-Astrain[1], David Sánchez-Porras[1], Óscar-Darío García-García[1], Agustín Janer[2], Emilio Esteban[2], M. José de Frutos[2], Javier Fernández[2], Víctor Carriel[1], Miguel Alaminos[1]

[1] Tissue Engineering Group, Department of Histology, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain

[2] Hispanagar SA, Burgos, Spain

Corresponding author's email: malaminos@ugr.es

BACKGROUND: Different agarose types have been used in tissue engineering (1,2), but the specific properties of each agarose type have not been elucidated. In the present work, we evaluated the in vivo biocompatibility of 5 types of agarose for use in tissue engineering.

METHODS: Agarose hydrogels were generated at the concentration of 3% and 0.3% using agaroses LM, D2LE, D1LE, MS8 and D5, and each biomaterial was subcutaneously grafted in Wistar rats. Then, the implant area was analyzed after 3 months of follow-up.

RESULTS AND DISCUSSION: Agaroses D1LE, D2LE and D5 at 3% tended to remain unaltered after 3 months, whereas agaroses LM and MS8 were partially reabsorbed at this time. At 0.3%, most grafted materials became reabsorbed, especially LM and MS8. Histologically, all agarose types were very biocompatible, without inflammation or side effects. Whilst agaroses D1LE and D5 became encapsulated by fibrous tissue, agaroses LM, MS8 and partially, D2LE, were progressively replaced by the host fibrous tissue with abundant collagen and fibroblasts, especially at the lowest concentration.

CONCLUSIONS: These results suggest that all agaroses were very biocompatible, and that LM and MS8 should be used when a rapid effect is sought, whereas D1LE and D2LE would be preferable for a long-time duration effect.

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Abstract **#1552**

SKIN ORGANOIDS RECAPITULATE SKIN MICROARCHITECTURE AND FUNCTIONAL ACTIVITY IN VITRO

Anastasiya Gorkun[P][1,2,3], Adam Jorgensen[1], Naresh Mahajan[1], Mingsong Wu[1], Sang Jin Lee[1], James Yoo[1], Anthony Atala[1], Shay Soker[1]

[1] *Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine*

[2] *FSBSI Institute of General Pathology and Pathophysiology*

[3] *Sechenov First Moscow State Medical University, Institute for Regenerative Medicine*

Corresponding author's email: stgork@gmail.com

To avoid the use of animals for drug and cosmetic testing, and to better model human skin diseases, numerous bioengineered human skin model in vitro were recently developed. In this study, we developed human skin tissue constructs (organoids) containing different skin cell types as a novel tool to recreate a human skin microarchitecture and restore native cell interactions. Cellular aggregates containing keratinocytes, melanocytes, fibroblasts, pre-adipocytes, endothelial and follicle dermal papillae cells self-organized into organoids and maintained long-term viability. Visual analysis demonstrated progressive maturation of the skin organoids towards uniform pigmentation up to day 21. Combined analysis of histology, scanning electron microscopy and immunohistology assay revealed the skin-specific organization of the organoids with layered microstructure, with a surface zone formed by epidermis cells (keratinocytes, melanocytes) and the central core formed by dermal (fibroblasts and follicle dermal papillae cells) and hypodermal cells (pre-adipocytes). The skin organoids also contained tubule-like structures in the central core formed by endothelial cells and showed high production of extracellular matrix proteins such as collagen III and laminin. Functional testing of the skin organoids in response of UVB exposure demonstrated converting retinol into retinoic acid and increased melanin synthesis. Ultimately, this technique provides an in vitro model of skin, and could be used as a platform for investigation of dermatopathologies, drug testing, and incorporation into body-on-a-chip platforms.

Keywords

skin organoids; pigmentation; ultraviolet B exposure

Abstract **#1553**

SILICON FLUORESCENT NANOPARTICLES AS MATERIALS THAT MODULATE THE INFLAMMATION AND PROMOTE THE M2A/M2C MACROPHAGE PHENOTYPES POLARIZATION.

Pedro Ulises Munoz-Gonzalez[1,2], Birzabith Mendoza-Novelo[2], Gerardo Gonzalez-Garcia[1]

[1] *División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Guanajuato, Mexico.*

[2] *División de Ciencias e Ingenierías, Universidad de Guanajuato, León, México.*

Corresponding author's email: pu.munozgonzalez@ugto.mx

Chronic wounds are characterized by a highly inflammatory environment that prevents healing. Macrophages are the key players in the inflammation since they polarize into two main phenotypes, the pro-inflammatory M1 (secrete the TNF-α, IL1β cytokines), and the anti-inflammatory M2 which can be classified into three subtypes: M2a (lymphocyte response, secrete IL10, TGF-β1), M2b (immunoregulation, secrete IL10, IL1β) and M2c (matrix remodeling, secrete IL10, TGF-β1). Silica nanoparticles have been shown to regulate the inflammatory response of macrophages, with the nanoparticle's small size and the presence of silicon implicated in the Nlrp3 inflammasome activation. To address this, silicon nanoparticles (SiNps) were synthesized from N-(2-aminoethyl)-3-aminopropyltrimethoxysilane. The nanoparticle size obtained by electronic microscopy was smaller than 10 nm, and the nanoparticles produced fluorescence under UV light. The main peak of 29Si RMN spectra at -73 ppm indicated that SiNps are composed of Si(-Si)4 molecules. To evaluate the macrophage cytocompatibility and the inflammatory response, five concentrations were tested (5, 10, 20, 40, 80 µg SiNps /mL) obtaining that all groups produced a similar metabolic activity compared to control. A concentration of 10µg/mL of SiNps down-regulated the production of the pro-inflammatory cytokines TNF-α and IL1β, and up-regulated the anti-inflammatory cytokines IL10 and TGF-β1. The SiNps 10 µg/mL concentration produced cytokines comparable to the IL4-stimulated anti-inflammatory control. These results suggest that SiNps can regulate inflammation in macrophages and have the potential to promote wound healing by polarizing M2c macrophages.

Keywords

Silicon fluorescent nanoparticles; Macrophage inflammation modulation; Chronic wound inflammation

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Abstract **#1554**

METABOLIC MODULATION AND DYNAMIC 3D CELL CULTURE AS DRIVING FORCES OF CARDIOMYOCYTE MATURATION IN A SCALE-UP PROCESS

Marta Paiva[1,2], Ana Filipa Louro[1,2], Claudia Correia[1,2], Marta Oliveira[1,2], Catarina Gomes[1,2], Kadla Rosholm[3], Sandra Wilson[3], Paula Alves[1,2], Margarida Serra[1,2]

[1] *IBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*

[2] *ITQB, Instituto de Tecnologia e Biológica António Xavier, Universidade Nova de Lisboa, Portugal*

[3] *Sophion Bioscience A/S, Ballerup, Denmark*

Corresponding author's email: mpaiva@ibet.pt

The implementation of robust methods for in vitro differentiation of human induced pluripotent stem cells into cardiomyocytes (hiPSC-CM) which produce cells that truly recapitulate the adult heart counterpart is an unmet need of pharma industry. Here, we describe our strategy to obtain more mature hiPSC-CM by combining 3D cell aggregation with metabolic modulation in dynamic suspension cultures.

Aggregation of hiPSC-derived cardiac progenitors was done in a scalable differentiation protocol yielding highly pure hiPSC-CM cultures, followed by metabolic maturation, in a galactose and fatty-acid supplemented medium (GalFA-M) (1,2).

Whole-transcriptome analysis showed that 3D hiPSC-CM aggregates cultured in GalFA-M had down-regulated glycolysis and lipid biosynthesis-related genes and enhanced expression of oxidative phosphorylation genes, compared to onset maturation. Ultrastructure images obtained by transmission electron microscopy highlighted the significant increase of mitochondrial density and intercellular junctions in the GalFA-M group. Nonetheless, calcium handling assays showed no maturation signs in the GalFA-M group. Sensitivity to doxorubicin and menadione was observed in a dose-response manner in hiPSC-CM aggregates cultured in GalFA-M, a deleterious effect abolished by antioxidant agents. Lastly, patch-clamp analysis suggested an increase of both sodium and calcium currents, which was more pronounced in the GalFA-M matured cells.

Our work demonstrates that combinatorial approaches where metabolic modulation and dynamic cultures are driving forces, will be fundamental for the recapitulation in vitro of the highly complex and controlled cardiac maturation process.

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Abstract **#1555**

IMPACT ON SUBSTRATE STIFFNESS AND TOPOGRAPHY ON NEURONAL DIFFERENTIATION

Sabrina KF Mattiassi[1], Ai Kia Yip[2], Anh Tuan Nguyen[3], Keng Hwee Chiam[2], Evelyn KF Yim$_P$[1]

[1] *University of Waterloo*

[2] *Bioinformatics Institute Singapore*

[3] *National University of Singapore*

Corresponding author's email: eyim@uwaterloo.ca

While many studies have investigated the effect of topography and stiffness on neuronal differentiation independently, few have investigated the combined effect. In vivo, cells are exposed to both cues simultaneously. Independent investigations have offered a plethora of information on cellular responses to biophysical cues, but a systematic investigation into the combined response could be important to understand the mechano-sensing mechanism, and to better tailor biomaterials for more precise control of neuronal differentiation. To study the combined effect, we have used polyacrylamide – 6-acrylamidohexanoic acid (PAA) hydrogels of varying stiffnesses (6 kPa – 110 kPa). These gels were patterned with anisotropic micropatterns of various dimensions using soft lithography, with surface-immobilized laminin for cellular adhesion. Mouse neuronal progenitor cells (mNPCs) were differentiated for two weeks, and immunofluorescent staining for TUJ1, MAP2 and GFAP was performed. Their lineage commitment, rate of differentiation and morphology on the gels were assessed.

To further understand the mechanisms and forces involved in the grating-induced cell elongation and differentiation, 3-dimensional (3D) cell traction stress measurements were made on the soft elastic PAA hydrogels with grating substrates. Using fibroblasts as model, we observed the cells forming protrusions in the grating grooves, and elongating and aligning parallel to the grating direction. The 3D traction stress measurements revealed that elongated fibroblasts on grating substrates exert anisotropic traction stresses, in the direction parallel to the grating direction. We propose that the increased cytoskeletal tension in the direction parallel to the grating would lead to polarized traction stresses which drive cell elongation.

Abstract **#1556**

ANTIMICROBIAL PEPTIDE IMMOBILIZATION OPENS THE DOOR FOR IMPROVED CHITOSAN-BASED DRESSINGS FOR CHRONIC INFECTED WOUNDS

Pedro M. Alves$_P$[1,2], Rúben Pereira[1], Natália Fagundes[3], Cátia Teixeira[3], Cristina C. Barrias[1,4], Paula Gomes[3], Fabíola Costa[1], M Cristina L Martins[1,4]

[1] *I3s, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

[2] *Faculdade de Engenharia, Universidade do Porto, Porto, Portugal*

[3] *LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal*

[4] *Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*

Corresponding author's email: pmalves@i3s.up.pt

Bacterial infection is often a hurdle in chronic wound resolution. To mitigate infection, antimicrobial peptides (AMP) have been seen as a solution to minimize the induction of bacterial resistance often associated with traditional antimicrobial formulations[1,2]. Peptides should be shielded from the harsh proteolytic environment in chronic wounds, which may be achieved by covalent immobilization[3,4]. The antibacterial effect of covalently immobilized AMP Dhvar5 has already been shown by our group using copper catalyzed azide-alkyne cycloaddition (CuAAC) [5,6]. However, the high quantity of peptide used (4% yield) constituted a potential barrier for future industrial scale-up. Herein, Dhvar5 was covalently immobilized onto chitosan using the highly reactive thiol-norbornene photoclick chemistry (TNPC). The aim is to efficiently improve the natural antimicrobial properties of chitosan in wound dressing applications. Successful AMP immobilization was extensively monitored by several techniques (1H-NMR, XPS, FTIR and Amino Acid Analysis). Dhvar5-chitosan films were produced by spin-coating and the viability of Staphylococcus epidermidis (ATCC 35984) was assessed by staining with BaclightTM LIVE/DEAD® kit (Syto9/Propidium Iodide). Dhvar5-Chitosan by TNPC led to the immobilization of 34 µmol Dhvar5/g modified chitosan (18.5% yield). Results suggest that Dhvar5-chitosan films exhibit enhanced anti-adhesive properties when compared to chitosan films, while also reducing bacteria viability. Therefore, this strategy not only increased the efficiency of Dhvar5 immobilization, but also preserved the antibacterial properties already observed for this AMP. Overall, Dhvar5-chitosan synthesized using TNPC exhibited promising antibacterial activity that may be extremely useful for the fabrication of wound dressings with improved ability to fight bacterial infection. Acknowledgments: SFRH/BD/145471/2019, POCI-01-0145-FEDER-031781.

Keywords

Antimicrobial peptides; Chitosan; Covalent immobilization

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Abstract **#1557**

DEVELOPMENT OF A LUNG-ON-A-CHIP MODEL FOR PRECLINICAL RESPIRATORY GAS ANALYSIS

Simon Heine$_P$[1], Linxiao Yang[1], Ralf Kemkemer[2,3], Petra Kluger[2]

[1] *Reutlingen Research Institute, Reutlingen University, Reutlingen, Germany*

[2] *Department of Applied Chemistry, Reutlingen University, Reutlingen, Germany*

[3] *Max Planck Institute for Medical Research, Heidelberg, Germany*

Corresponding author's email: Simon.Heine@Reutlingen-University.de

INTRODUCTION: The measurement of volatile organic compounds (VOCs) or drug concentrations in the exhaled air represents a promising approach in individualized medicine. The aim of the project is to establish a lung microchip for its use in preclinical respiratory gas analysis.

METHODS: Endothelial cells were cultured in MCDB131 medium and epithelial cells were cultured in RPMI medium, in cell culture dishes, ThinCerts™ and on PDMS. For chip fabrication, PDMS was poured into a 3D printed master with the negative chip structures. Alginate was covalently bound on the PDMS to create a hydrophilic surface. Barrier function was assessed via immunofluorescence staining, TEER measurement and via permeability assays. Propofol was sampled via solid phase microextraction (SPME). Subsequent analysis was carried out using GC-MS.

RESULTS: A lung-on-a-chip was constructed, consisting of a liquid and air microchannel, which are separated by a semipermeable membrane. Associated microfluidic was established. Propofol dissolved in medium could be detected in gas phase using SPME-GC-MS. Absorption of propofol by the lung-chip was reduced by alginate-coating. The alveolar-co-culture model was viable for two weeks in air-liquid-culture and sustained good barrier functions. Chip materials were biocompatible.

DISCUSSION & CONCLUSIONS: An air-liquid-co-culture model was successfully established representing key features of an alveolar barrier, enabling the investigation of alveolar drug passage. Proof-of-principle of respiratory gas analysis of a VOC, was shown by semi-quantification of propofol. This lung-on-a-chip model is highly relevant for many applications that include quantitative investigations using organ-on-a-chip systems.

ACKNOWLEDGEMENTS: Financial support was received from the Baden-Württemberg Stiftung gGmbH.

Keywords

Lung-on-a-chip; Respiratory gas analysis; Volatile organic compound quantification

Abstract **#1558**

A BIOPRINTED IN VITRO MODEL OF THE OSTEOCHONDRAL INTERFACE

Megan Cooke$_P$[1,2], Samantha Ozere[1], Thierry Pauyo[2], Lisbet Haglund[1], Derek Rosenzweig[1]

[1] *McGill University*

[2] *Research Institute of the McGill University Health Centres*

Corresponding author's email: megan.cooke.research@gmail.com

Osteoarthritis (OA) is a painful and disabling condition driven by degradation of articular cartilage and underlying bone. OA is a leading cause of painful disability and loss of work worldwide. The contribution of bone and cartilage phenotypes and exact mechanisms of degeneration are still being elucidated. In this study we used bioprinting to produce in vitro models of the human osteochondral interface with spatially defined bone-like and cartilage-like regions. Bioprinting has grown rapidly over the past decade and is a powerful tool for medium-high throughput production of 3D in vitro models. These enable more physiologically relevant environments to screen potential therapeutics compared to traditional 2D culture. Using an alginate-gelatin bioink (with nanocrystalline hydroxyapatite for the bone-like region) we printed constructs with patient-derived osteoblasts and chondrocytes for bone and cartilage-like regions respectively. As the materials used in both regions are similar, there was very good integration at the interface and they did not delaminate. Initial experiments showed that in appropriate culture media, tissue specific extracellular matrix was formed with high cell viability. Furthermore, using chondroadherin peptides specific for integrin α2β1 binding, in conjunction with TGF-B1, we found that production of proteoglycan-rich matrix was increased in chondrocyte pellet cultures and within the chondral region of the bioprinted model. Ongoing experiments are currently probing osteogenic and chondrogenic gene expression markers in respective compartments to assess stability of the model over 28 days of culture. This model may provide a novel, reproducible human tissue platform for screening therapeutics and assessing mechanisms of osteochondral pathology.

Abstract **#1559**

ANGIOGENIC AND LYMPHANGIOGENIC POTENTIAL OF FIBRIN-AGAROSE MODELS OF BIOARTIFICIAL HUMAN SKIN. AN IN VIVO STUDY

Miguel Alaminos$_P$[1], Cristina Blanco-Elices[1], David Sánchez-Porras[1], Óscar-Darío García-García[1], Olimpia Ortiz-Arrabal[1], Gloria Carmona[2], Fernando Campos[1], Ingrid Garzón[1], Antonio Campos[1], Miguel Ángel Martín-Piedra[1]

[1] *Tissue Engineering Group, Department of Histology, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain*

[2] *Red Andaluza de Diseño y Translación de Terapias Avanzadas (RADYTTA), Sevilla, Spain*

Corresponding author's email: malaminos@ugr.es

Objective: The aim of this work is to evaluate the angiogenesis and lymphangiogenesis potential of a fibrin-agarose model of bioartificial human (BHK) skin (1,2) once grafted in vivo.

Methods: First, BHK was generated fulfilling all requirements of the national medicines agency using nanostructured fibrin-agarose biomaterials containing dermal fibroblasts within and a stratified epithelium on top. BHK was grafted in vivo, and small tissue biopsies from the grafting area were taken after 1 month of follow-up. Then, the presence of blood and lymphatic vessels was assessed by CD31, SMA and Podoplanin immunohistochemistry.

Results and discussion: Results showed that the BHK became properly integrated at the host site. Grafted BHK was capable to regenerate mature SMA and CD31-positive blood vessels after 1 month. Podoplanin expression was found in some thin vessels, suggesting that artificial autologous skin could also induce the formation of mature lymphatic vessels. These results demonstrate that BHK has in vivo angiogenic and lymphangiogenic potential. In turn, neovessels might be responsible for the excellent biointegration showed by BHK.

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Abstract **#1560**

REPRODUCIBLE TISSUE-SPECIFIC BIOINKS BASED ON DECELLULARIZED EXTRACELLULAR MATRIX: MIMICKING THE NATIVE MICROENVIRONMENT TO MODULATE CELL BEHAVIOR

Tácia T.A. Liguori[P][1], Renata F Bombaldi de Souza[1], Fernanda C Bombaldi de Souza[1], Leonardo O.P. Longo[1], Emerson G Moretto[1], Gabriel R Liguori[1]

[1] *TissueLabs SAGL*

Corresponding author's email: gabriel@tissuelabs.com

INTRODUCTION: Bioprinting requires the use of hydrogels together with cells to produce 3D tissue constructs. Promising biomaterials that can be used as hydrogels for 3D bioprinting are extracellular matrix (ECM) proteins. Some bioinks are made of collagen type I, some contain RGD motifs, and some are added of laminin, fibrinogen/thrombin, among other ECM proteins. However, the extracellular microenvironment of the cells is exceptionally more complex, containing hundreds to thousands of unique proteins. The presence and concentration of such proteins are different for each distinct tissue. Thus, the use of a hydrogel containing the whole protein content of the native ECM would be an ideal candidate for constituting truly representative bioinks. METHODS: We developed a method to produce scalable and reproducible tissue-specific hydrogels based on decellularized extracellular matrix. For 3D bioprinting purposes, these hydrogels can be mixed with cells to compose a thermocrosslinkable bioink. Alternatively, a photocrosslinkable version of the hydrogels is also available. RESULTS: ECM hydrogels were produced from 15 different tissues: adipose tissue, bone, brain, cartilage, colon, intestine, kidney, liver, lung, skeletal muscle, myocardium, skin, spleen, stomach, and blood vessels. These hydrogels demonstrated promising biochemical and biomechanical properties for 3D bioprinting applications. The conventional version can be used with the freeform reversible embedding of suspended hydrogels (FRESH) method, while the photocrosslinkable version can be used for direct printing on culture dishes/plates. CONCLUSION: Tissue-specific bioinks based on ECM are a promising alternative for mimicking the native microenvironment in 3D bioprinted tissue constructs.

Keywords

Extracellular matrix; Tissue-specific; Hydrogels

Abstract **#1561**

BIOFABRICATION OF BIOENGINEERED ORAL MUCOSA SUBSTITUTES WITH ENHANCED PROPERTIES FOR USE IN ORAL AND MAXILLOFACIAL SURGERY

Óscar-Darío García-García[P][1], Carmen Miranda-Fernández[1], Cristina Blanco-Elices[1], Jesús Chato-Astrain[1], David Sánchez-Porras[1], Olimpia Ortiz-Arrabal[1], Ismael A. Rodríguez[2], Fernando Campos[1], Ingrid Garzón[1], Miguel Ángel Martín-Piedra[1]

[1] *Tissue Engineering Group, Department of Histology, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain*

[2] *Universidad Nacional de Córdoba, Argentina*

Corresponding author's email: malaminos@ugr.es

INTRODUCTION: Development of viable substitutes of oro-maxillo-facial tissues (OMFT) depends on the capability to generate a biomimetic ECM ex vivo. As mesenchymal stem cells (MSC) are able to synthetize numerous trophic and inductive factors, our hypothesis is that the use of secretome generated by MSC could contribute to optimize OMFT primary cell cultures.

METHODS: Human oral mucosa fibroblasts were cultured in a medium enriched with secretome derived from three MSC: dental pulp, Wharton's jelly and bone marrow. Cell viability was assessed by WST-1 and Live/Dead, whereas the synthesis of several proteins playing a role in ECM function was evaluated using a human proteins array.

RESULTS: The use of the enriched medium did not alter cell viability as compared to controls. Then, we found that several proteins were expressed as similar levels in both groups, including several metaloproteases, although an increase of proteins with a role in ECM composition and wound healing, such as TIMP1 (1), was detected.

CONCLUSIONS: MSC secretome may enhance oral mucosa fibroblasts to the synthesis and maturation of extracellular matrix of bioengineered stromas. Optimization of extracellular matrix synthesis play a key role in the development of tissue engineered oral mucosa substitutes.

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Abstract **#1562**

PROSTATE CANCER ORGANOIDS: 3D TUMOR MINIATURES AS FRONTLINE MODELS FOR PERSONALIZED MEDICINE

Soraia Fernandes$_P$[1], Marco Cassani[1], Jorge Oliver-De La Cruz[1], Helena Ďuríková[1], Petr Filipenský[2], Giancarlo Forte[1]

[1] *International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

[2] *St. Anne's University Hospital, Brno, Czech Republic*

Corresponding author's email: soraia.fernandes@fnusa.cz

Due to the possibility to recreate more accurate in vitro physiological systems, organoid technology has become very popular among researchers over the past decade. Organoids can be defined as small 3D copies of their in vivo tissue of origin, realistically reproducing its architecture and function. Therefore, they have been recognized as valuable pre-clinical models in the field of cancer research. (1,2)

When it comes to prostate cancer, it is particularly challenging to establish a cellular disease model mainly due to the great interpatient therapy response variability. Although prostate cancer organoids (PCOs) have been obtained, at a low success rate, from metastatic tissue or circulating tumor cells, the isolation of organoids from primary prostate cancer is still missing. (3-5)

In this perspective, we have developed a suitable protocol, with an optimized media formulation, for isolating PCOs directly from primary tumor tissue. Comparing with non-tumorigenic organoids, PCOs show an invasive phenotype in 3D culture resembling the in vivo tumor histological features. Moreover, the expression of tumor specific markers and multicellular composition of the PCOs, containing both epithelial and mesenchymal cells, were confirmed, by immunohistochemistry, to be similar to the parental tissue. The validation of this protocol will allow the establishment of patient specific cancer models for testing personalized therapies and prevent cancer propagation before it achieves a more advanced stage (i.e. metastasis).

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Abstract **#1563**

BMSCS-LADEN NITRIC OXIDE RELEASING HYDROGEL ACCELERATES WOUND HEALING IN DIABETES INDUCED RABBITS

Rashid Ahmed$_P$[1], Muhammad Tariq[2], Robin Augustine[1], Alap Ali Zahid[1], Anwarul Hasan[1]

[1] *Department of Mechanical and Industrial Engineering, College of Engineering, Qatar University– 2713, Doha, Qatar*

[2] *Department of Biotechnology, Mirpur University of Science and Technology, Mirpur 10250, AJK, Pakistan*

Corresponding author's email: rashid.ahmed@qu.edu.qa

Impaired diabetic wounds are one of the major pathophysiological complications caused by persistent microbial infections, prolonged inflammation, and insufficient secretion of angiogenic promoting growth factors [1]. Here, we report the development of nitric oxide (NO) releasing S-nitroso-N-acetyl-pencillamine (SNAP) loaded chitosan/polyvinyl alcohol hydrogel and its application to precondition bone marrow stem cells (BMSCs) for accelerating the healing of the diabetic wounds. The study exhibited that NO-releasing hydrogels significantly increase the cell viability and cell proliferation of hydrogen peroxide (H2O2) pretreated BMSCs depicting its cytoprotective activity on BMSCs which was confirmed through manifold increase in the gene expression of B-cell lymphoma 2 (Bcl-2), stromal cell-derived factor 1alpha (SDF-1α), proliferating cell nuclear antigen (PCNA) and vascular endothelial growth factor (VEGF). Furthermore, the SNAP loaded hydrogel showed continuous cell proliferating activity for six days due to the continuous of NO from the hydrogel samples. Wound healing studies on diabetes-induced rabbits showed that the application of SNAP preconditioned BMSCs first and then NO-releasing hydrogels significantly speed up the healing process compared to the control groups. The wound healing potential of BMSCs plus NO-releasing hydrogel was further validated by improved collagen deposition and epithelial layer formation by histopathological examinations as well as upregulation of VEGF and SDF-1α biomarkers through gene expression analysis assays [2]. The results of this study displayed that the application of BMSCs and NO-releasing hydrogel can help in faster regeneration and repair of damaged tissues. Therefore, BMSCs plus NO-releasing hydrogels can be very useful for the treatment of diabetic wounds.

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Abstract **#1564**

PRE-VASCULARIZED ARTIFICIAL ORAL MUCOSA USING HUMAN DENTAL PULP STEM CELLS

Jesús Chato-Astrain[P][1], Cristina Blanco-Elices[1], Olimpia Ortiz-Arrabal[1], David Sánchez-Porras[1], Salvador Oyonarte[1,2], Miguel Alaminos[1], M. Carmen Sánchez-Quevedo[1], Ricardo Fernández-Valadés[3], Miguel-Ángel Martín-Piedra[1], Ingrid Garzón[1]

[1] Tissue Engineering Group, Department of Histology, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain

[2] Red de Medicina Transfusional, Células y Tejidos de Andalucía

[3] Division of Pediatric Surgery, Univ. Hospital Virgen de las Nieves, Granada, Spain

Corresponding author's email: malaminos@ugr.es

BACKGROUND: Regeneration of oral mucosa depends on a proper vascular supply. In this regard, human dental pulp stem cells (DPSCs) are known to possess strong pro-angiogenic potential (1). In the present work, we generated and evaluated a tissue-engineered oral mucosa constructs containing pre-vascular components to determine its biointegration potential once grafted in vivo.

METHODS: Nanostructured oral mucosa constructs based on fibrin-agarose scaffolds were developed (2), seeded with DPSCs, and cultured in a preinductive medium con-taining VEGF. Angiogenesis potential was evaluated ex vivo and in vivo using flow cy-tometry and immunohistochemistry.

RESULTS: First, we found that cells tended to decrease the expression of stem cell markers upon ex vivo induction (CD105, CD90 and CD73), with an increase of vascu-lar differentiation markers such as VEGF, endothelin-1, angiogenin-1 and endostatin. Then, in vivo grafting in nude mice resulted in an increased number of blood vessels with positive expression of CD31 and vWF, coinciding with a process of extracellular matrix regeneration.

CONCLUSIONS: These results confirm that DPSCs have pro-angiogenic and endothe-lial-like differentiation potential ex vivo an in vivo and support the use of pre-vascularized human oral mucosa constructs to increase tissue regeneration in patients.

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Abstract **#1565**

TWO PHOTON IMAGING – HISTOLOGICAL RESOLUTION WITH NETWORK INFORMATION

Vytautas Kučikas$_P$[1], Maximilian Werner[2], Stefan Jockenhoevel[2], Thomas Schmitz-Rode[2], Marc A.M.J. van Zandvoort[1]

[1] *Institute for Molecular Cardiovascular Research (IMCAR), Interdisciplinary Centre for Clinical Research, RWTH Aachen University, Aachen, Germany*

[2] *Department of Biohybrid and Medical Textiles (BioTex), AME - Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany*

Corresponding author's email: vkucikas@ukaachen.de

In biological and medical research histology seems to be the ultimate tool for registering structural composition of the specimen. Even though it is rendered as a "gold standard", a huge drawback is inevitably connected to the preparation process, namely, destruction of living tissue. Consequently, histology does not allow to assess changes of state of a specific specimen over time and therefore has no role in monitoring of in vivo experiments.

In tissue engineering experiments, extracellular matrix formation ("networking") of the growing cells is crucial information, which represent tissue maturation and biomechanical stability. We present the application of two photon microscopy for registering the network structure of smooth muscle cells grown in fibrin gel, at the same time registering all the information accessible by histology. Our results show that histological slicing might be misleading in terms of quantification of the compounds (nuclei, SMA, Collagen), as the amount of cell structural proteins varies dramatically in between randomly chosen slices. Such errors are easily avoided by using two photon microscopy, capable of registering the whole 3D cellular and fibrous network.

Furthermore, we report application of two photon endoscopic probe in tissue engineering experiments, enabling longitudinal in vitro (and in vivo) structural assessment. This application proves that such endoscopic technology can be successfully used to monitor maturation in in-vitro tissue engineering applications and is compatible with strict sterility, temperature, and gas composition requirements.

Abstract **#1566**

A 3D-PRINTED BONE IMPLANT FOR HIP DYSPLASIA TREATMENT: COMBINING SHAPE AND MECHANICAL FUNCTION IN A FULLY RESORBABLE IMPLANT

Nasim Golafshan[P][1,2], Koen Willemsen[1], Harrie Weinans[1], Bart C H van Der Wal[1], Jos Malda[1,2,3], Miguel Castilho[1,2,4]

[1] *Department of Orthopaedics, University Medical Center Utrecht, GA Utrecht, The Netherlands;*

[2] *Regenerative Medicine Utrecht, Utrecht, The Netherlands*

[3] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands*

[4] *Orthopaedic Biomechanics, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands*

Corresponding author's email: n.golafshan@umcutrecht.nl

Osteoarthritis (OA) is a painful and debilitating condition that affects over 40 million people just in Europe (1). Together with the knee, the hip is one of the joints most frequently involved in OA. One of the two main causes for hip OA is hip dysplasia (HD) (2). HD is characterized by the instability of the hip joint and is caused by incomplete coverage of the femoral hip by the acetabulum. In this study, we have developed a mechanical competent and fully resorbable magnesium phosphate (MgP) - based implant to enlarge the acetabulum. The appropriate design was obtained from CT-scans of eight dog patients and fabricated using a room-temperature extrusion-based process (3). The mechanical compatibility of the implant as well as stability and optimal femoral coverage in the load-bearing quadrant of dog model is being investigated using a custom-built mechanical set-up simulating canine hip joint forces. Our results demonstrate that the 3D-printed implant design adapted well to the acetabulum of canine dysplastic hips, while the the flexibleMgP material composition supported fixation with metallic screws. Implants were able to withstand a maximum shear force of 900Nwithout failure and plastic deformation, which confirmed a proper fixation of the implant to the defect side. . Currently, implant mechanical performance under accelerated in vitro degradation and physiological loading is being performed. Preclinical evaluation in a canine model is planned to demonstrate safety of the proposed implant to treat hip dysplasia.

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Abstract **#1568**

A NOVEL MICROCHIP FOR GENETIC HETEROGENEITY OF CANCER TISSUES

Anwarul Hasan$_P$[1], Rashid Ahmed[1], Nasrin Mesaeli[2], Irfan S. Ahmad[3], Rashid Bashir[3]

[1] *Department of Mechanical and Industrial Engineering, College of Engineering, Qatar University– 2713, Doha, Qatar*

[2] *Department of Biochemistry, Weill Cornell Medicine in Qatar, Doha, Qatar*

[3] *Nick Holonyak Micro and Nanotechnology Lab, University of Illinois, Urbana-Champaign, Urbana, IL 61801, USA*

Corresponding author's email: ahasan@qu.edu.qa

Early detection of cancers can make preventative measures possible (1). However, the current methods used to diagnose various types of cancers have severe limitations, resulting not only in missed cases but also in the diagnosis of a high rate of false positives. Genetic heterogeneity has been demonstrated to play a crucial role in all cancers, between individual tumors of the same histopathologic subtype (2). Thus, we developed a novel microchip-based spatial gene expression analysis assay for lung cancer detection along with the heterogeneity of their expression levels in these tumors. We achieved this by performing the analysis using a rapid technique that performs on-chip picoliter real-time reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) reactions on a histological tissue section without any analyte purification while maintaining the native spatial location of the nucleic acid molecules. Using our molecular histopathology technique, we will analyze resistin like alpha (Retnela) gene amplification in lung cancer biopsy tissues on a microchip platform. Lung cancer tissue from transgene mice was harvested to visualize the spatial variation of Retnela mRNA using our technique respectively. We also demonstrated this method by amplifying the Retnela messenger RNA (mRNA) with 100µm spatial resolution and by visualizing the variation in threshold times across the tissue. In future studies, the on-chip reaction will be validated through label-free Fourier transform infrared (FT-IR) spectroscopic imaging, which classifies tumor histology and its spatial genetic heterogeneity in the tissue section.

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Abstract **#1570**

COMPOSITION-STRUCTURE-PROPERTY RELATIONSHIP IN THREE- DIAMETER DECELLULARIZED PORCINE ESOPHAGEAL MATRIX

Paola GarcíaP[1], Adriana Orozco[1], Metzeri Montes[1], Birzabirh Mendoza[1]
[1] *División de Ciencias de Ingeniería; Universidad de Guanajuato, León, México.*
Corresponding author's email: ep.garciatejada@ugto.mx

Xenogeneic biomaterials that retain the composition, bioactivity, architecture, and mechanics of the native tissue can be an alternative for the management of defects in esophageal tissue. Here, decellularized porcine esophageal tubular matrix of three different diameters was derived from piglets' tissue at 1 (D1), 21 (D21) and 45 (D45) days after birth. The removed DNA (99.1, 97.9 and 95.5% for D1, D21 and D45, respectively) and the absence of nuclear structures (H&E staining) were indicators of effective cell removal. Furthermore, the esophageal matrix structural network and its tensile behavior were preserved after decellularization, according to its characterization as a multilayer material or as separate muscular and submucosa layers. The content of residual GAGs (48.6, 74 and 70.2%) and the extracted residual fibronectin (69.7, 74.2, 86.3%) for D1, D21 and D45, suggests the conservation of bioactive components in the matrix. We propose that the decellularized esophageal tubular matrix serve as a template capable of coating it with extracellular matrix gel and therapeutics (epoxyeicosatrienoic acids, cells) as a strategy to enhance bioactivity in the search of restoration of injured esophageal tissue.

Keywords
HIDROGEL COATED DECELLULARIZED TISSUE ; ESOPHAGEAL SCAFFOLD; ESOPHAGEAL REPLACEMENT

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Abstract **#1571**

MECHANISTIC UNDERSTANDING OF BLOOD-INDUCED CHONDROCYTE DEATH

Andy Lee[P][1], Lianna Gangi[1], Fereshteh Zandkarimi[2], Brent Stockwell[2,3], Clark Hung[1,4]

[1] *Department of Biomedical Engineering, Columbia University in the City of New York*

[2] *Department of Biological Sciences, Columbia University in the City of New York*

[3] *Department of Chemistry, Columbia University in the City of New York*

[4] *Department of Orthopedic Surgery, Columbia University in the City of New York*

Corresponding author's email: jl3924@columbia.edu

Introduction. Hemophilic arthropathy is a debilitating disease due to recurrent joint bleeding and cartilage degeneration [1,2]. Ferroptosis, an iron-dependent mode of regulated cell death, may contribute to tissue damage via lipid peroxidation and altered lipid profiles [3]. We assessed the cytotoxic effects of isolated red blood cells (RBC) on human articular chondrocytes as well as changes in lipid profiles as an indicator of ferroptotic mechanisms.

Methods. Cultured human chondrocytes were treated with intact and lysed RBCs (0-50% v/v) as well as soluble hemoglobin (0-10 μM) for 4 days. Cell viability was visualized with calcein-AM (live) and ethidium homodimer-1 (EtH-1, dead). Lipidomics assessment was performed on isolated lipids and lipid species were identified using METLIN. Lipid peroxidation was detected using a BODIPY 581/591 C-11 Sensor. Data were analyzed with ANOVA with Tukey post-hoc tests ($\alpha < 0.05$).

Results and Discussion. Chondrocyte viability was reduced in all treatment groups compared to control in a dose-dependent manner. EtH-1 was most strongly expressed with RBC lysate, followed by hemoglobin and intact RBCs, suggesting RBC intracellular constituents (e.g., iron) may drive chondrocyte death. Lipidomics assessment revealed upregulated levels of fatty acid and ceramides, which have been implicated in lipotoxicity, apoptosis, matrix homeostasis disruption, and down-regulation of type II collagen in articular cartilage [4]. Lipid peroxidation, a hallmark feature of ferroptosis, was also significantly elevated in RBC-treated groups. Understanding mechanisms of blood-induced cell death can lead to development of chondroprotective therapeutics for patients with chronic conditions (e.g., hemophilia) and acute conditions (e.g., articular fractures).

Keywords

Hemarthrosis; Ferroptosis; Chondrocyte

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Abstract **#1573**

CHARACTERIZATION OF USPIO-PLGA SCAFFOLD DEGRADATION AND TISSUE REMODELING IN BIOHYBRID VASCULAR PROSTHESIS USING 7T MRI

Elena Rama-P[1]

[1] *Department of Experimental Molecular Imaging*

Corresponding author's email: erama@ukaachen.de

The longitudinal in-vivo monitoring of remodeling processes and pathological dysfunctions of implanted vascular prosthesis remains challenging [1]. This obstacle might be overcome by the integration of contrast agents into the main components of the vascular prosthesis structure [2]. In our study, we developed biohybrid vascular grafts with polyvinylidene fluoride (PVDF) and poly(lactic-co-glycolic acid) (PLGA). PLGA is the biodegradable component of the textile scaffold, which allows cell attachment and colonialization. Unfortunately, PLGA can hardly be visualized in-vivo. Therefore, in this study, we aimed at labeling PLGA fibers with ultra-small superparamagnetic iron oxide nanoparticles (USPIOs) to visualize their presence and degradation by MRI. PLGA fibers were embedded in fibrin gel and incubated at 37°C for 8 weeks and at 70°C for 3 weeks. USPIO-PLGA fibers combined with PVDF were incubated at 37°C in PBS for 3 weeks. With the degradation of the PLGA fibers, which was faster at higher temperatures, there was a strong increase in T2 and T2* relaxation times, which can be explained by the release of the USPIO. Then, the final textile structure (PVDF-PLGA-USPIOs) was molded in fibrin gel containing smooth-muscle and endothelial cells. Here, the degradation of the PLGA fibers was much faster due to the presence of cells. Furthermore, it was shown by immunofluorescence microscopy, that the degraded PLGA fibers were replaced by elastin, which was produced by the smooth-muscle cells. In conclusion, we could establish an MR imaging approach to non-invasively assess the degradation of PLGA scaffolds and could confirm its replacement by endogenous cellular matrix.

Keywords

Biohybrid vascular graft; Magnetic resonance imaging; ultrasmall superparamagnetic iron oxide nanoparticles

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Abstract **#1574**

HISTOLOGICAL PROPERTIES AND BIOCOMPATIBILITY OF NOVEL ACELLULAR PERIPHERAL NERVE ALLOGRAFTS

Óscar-Darío García-García[P][1], David Sánchez-Porras[1], Marwa El-Soury[2], Jesús Chato-Astrain[1], Stefania Raimondo[2], Fernando Campos[1], Salvador Oyonarte[1,3], M. Carmen Sánchez-Quevedo[1], Stefano Geuna[2], Víctor Carriel[1]

[1] Tissue Engineering Group, Department of Histology, University of Granada and Instituto de Investigación Biosanitaria ibs.GRANADA, Spain

[2] Department of Clinical and Biological Sciences, University of Torino, Italy

[3] Red de Medicina Transfusional, Células y Tejidos de Andalucía

Corresponding author's email: malaminos@ugr.es

Introduction: Decellularization may offer the possibility of obtaining acelular nerve allografts for use in tissue engineering. The aim of this study was to develop a novel chemical-enzymatic decellularization method (CE) for nerve decellularization.
Methods: Rat sciatic nerves were decellularized with the CE method (distilled water, triton X-100, SDS, SDC and nucleases) and compared with the previously-described Sondell (SD) technique (1). Afterwards, the ECM was evaluated by histological and ultrastructural analyses and biocompatibility was determined ex vivo by culturing adipose-derived mesenchymal cells on the surface of the decellularized nerves.
Results: Histological analysis demonstrated a complete removal of cell nuclei in CE and SD. Cells cultured on both models showed high viability, especially in CE. Picrosirius red staining demonstrated a good preservation of the nerve collagen network in CE and SD, whereas alcian blue revealed a substantial loss of glycosaminoglycans in SD, but not in CE. At the ultrastructural level, the ECM was properly preserved and well-organized.
Discussion & conclusion: The CE method resulted in a better preservation of the structure and composition of the ECM components, with good biocompatibility, supporting its potential usefulness in nerve repair.
Funding: Supported by Grants FIS PI17/393 and PI20/318, Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), co-financed by FEDER; grant P18-RT-5059 from Consejería de Economía, Conocimiento, Empresas y Universidad, Junta de Andalucía, Spain; and Grant A-CTS-498-UGR18, Programa Operativo FEDER Andalucía 2014-2020 and University of Granada, Spain.

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Abstract **#1575**

A DISTINCT PEPTIDE SIGNATURE FOR REMODELING AFTER MYOCARDIAL INFARCTION IN A MOUSE HEART

Charles Van Asschep[1], Stephanie Mezger[1,2,3], Alma Mingels[2,3], Otto Bekers[2,3], Paula Da Costa Martins[3,4], Ron Heeren[1], Berta Cillero Pastor[1]

[1] The Maastricht MultiModal Molecular Imaging Institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, The Netherlands

[2] Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, The Netherlands

[3] CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

[4] Department of Molecular Genetics, Faculty of Sciences and Engineering, Maastricht University, Maastricht, The Netherlands

Corresponding author's email: c.vanassche@maastrichtuniversity.nl

Introduction: Myocardial infarction (MI) remains the most common type of cardiovascular disease and the main cause of death worldwide. MI leads to myocardial ischemia, subsequent molecular changes, inflammatory pathway activation, and remodeling. In this project we aim to find specific peptide signatures, linked to cardiac remodeling in the mouse heart after MI.Methods: Mice susceptible for cardiac ischemic injury (miR-216a knock-out in a BL6CBAF1 background) were subjected to MI by permanent ligation of the left coronary artery or sham surgery. 4 weeks after surgery, the animals were sacrificed and the hearts were excised and formalin fixed paraffin embedded. 4µm thick transverse sections underwent on-tissue digestion before peptide analysis using MALDI-MSI at 50µm raster size on a RapifleX tissueTyper (Bruker, Bremen, Germany). Afterwards, sections were stained using H&E or Sirius Red.Results: Probabilistic latent semantic analysis revealed peptides belonging to haemoglobin in blood (m/z 1529.73) and actin in ventricles (m/z 976.45 and 1198.70) as the biggest contributors in components 1 and 2, respectively. Other discriminating m/z-values are still unidentified. Component 5 highlighted peptide masses co-localizing with the affected area in the heart as assigned by Sirius Red. Further spectral clustering analysis suggested a decrease of m/z 1906.84 and 2093.11 as compared to the unaffected area.Conclusion: The peptide profiles found for different regions in the heart might help to understand the mechanism underlying remodeling after MI. Validation of the results will be done using another MI mouse model and a MALDI-MSI guided laser capture microdissection proteomics workflow for additional peptide identification.

Keywords

Myocardial infarction; MALDI-MSI; Peptide signature

Abstract **#1577**

IN VITRO STUDIES ON THE BIOCOMPATIBILITY OF PCL SCAFFOLDS WITH COLLAGEN COATING

Anke Bernstein[P][1], Lucas Weingärtner[1], Pia Schilling[1], Michael Seidenstücker[1]

[1] *G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Medical Center - Albert-Ludwigs-University of Freiburg, Faculty of Medicine, Albert-Ludwigs-University of Freiburg, Freiburg, Germany.*

Corresponding author's email: anke.bernstein@uniklinik-freiburg.de

Various factors, including cells, biomaterials, cell and tissue culture conditions, play a crucial role in tissue engineering. The in vivo environment of the cells exerts complex stimuli on the cells and thus has a direct influence on cell behavior, including proliferation and differentiation. In order to create suitable replacement or regeneration procedures for human tissue, the conditions of the cells' natural environment should therefore be well imitated. Therefore, current research is trying to develop 3-dimensional scaffolds that can elicit appropriate cellular responses and thus help the body to regenerate or replace tissue.

Material and methods: The 3D biolplotter was used to produce the scaffolds. The printed polycaprolactone scaffolds were coated with type I collagen for improved cell-scaffold interaction. Subsequently, three different cell types (human MSC, MG63 and MLO-Y4) were cultivated on the scaffolds and the biocompatibility of the scaffolds was investigated using various tests (WST, LDH, live/dead assay). The test duration was 3, 7 and 10 days.

Results: Reproducible scaffolds made of polycaprolactone could be printed. In addition, a coating process with collagen was developed, which significantly improved the cell-caffold interaction. The cell experiments proved that the cells adhere and proliferate very well to the scaffolds. In the MSC and MG-63, it was also observed that the cells colonize the entire available scaffold surface by spanning the pores. Cell growth within the scaffolds was also examined. MLO-Y4 cells can interact with the collagen coating and migrate through it. All cells showed an increasing proliferation rate. The cytotoxicity was negligible.

Keywords

PCL scaffold; coating with collagen; biocompatibility

Abstract **#1579**

HUMAN-ON-A-CHIP SYSTEMS FOR EFFICACY AND TOXICITY DETERMINATION IN CANCER AND RARE DISEASE DRUG DISCOVERY APPLICATIONS

James Hickman[P][1,2]

[1] *University of Central Florida, NanoScience Technology Center*

[2] *Hesperos, Inc*

Corresponding author's email: jhickman@ucf.edu

We have been constructing human-on-a-chip systems for toxicology and efficacy with up to 6 organs and have demonstrated long-term (>28 days) evaluation of drugs and compounds, that have shown similar response to results seen from clinical data or reports in the literature. Application of these systems for ALS, Alzheimer's, rare diseases, diabetes, and cardiac, immune and skeletal muscle mechanistic toxicity will be described. These models utilize a pumpless platform with serum free recirculating medium that is a low volume system that can evaluate parent compounds as well as metabolites if the liver is included. We have constructed stem cell-based, human-on-a-chip systems demonstrating long-term physiology (>28 days) in configurations of up to five organs [1]. Acute and chronic compound testing in systems has generated drug efficacy and safety responses similar to those seen in clinical data or reports from literature [2]. Concurrent measurement of both efficacy and toxicity can also be done in the same system for therapeutic index estimation for chemotherapeutics [3]. We will describe HoaC systems composed of liver, cardiomyocytes, skeletal muscle myotubes, motoneurons and a functional, rudimentary kidney module in configurations relevant for parent compound and metabolite efficacy and safety testing. We have recently published a paper that described a multi-organ innate immune system that was able to reproduce the the pro-inflamatory and restorative phenotypes from macrophages [4]. This talk will also give results of six workshops held at NIH to explore what is needed for validation and qualification of these new systems.

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Abstract **#1580**

GELATIN AND HYALURONIC ACID-BASED INTERPENETRATING POLYMER NETWORK HYDROGEL AS AN EXTRUDABLE BIOINK FOR TISSUE ENGINEERING APPLICATIONS

Resmi Anand$_P$[1,2,3], Paulo Alexandre Amorim[1,2], Mehdi Salar Amoli[2], Liesbet Geris[1], Veerle Bloemen[1,2]

[1] *Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, O&N 1, Herestraat 49, 3000 Leuven, Belgium*

[2] *Materials Technology TC, Campus Group T, KU Leuven, Andreas Vesaliusstraat 13, 3000 Leuven, Belgium*

[3] *Inter University Centre for Biomedical Research and Super Speciality Hospital, Mahatma Gandhi University Campus at Thalappady, Kottayam, Kerala-686009, India*

Corresponding author's email: Veerle.bloemen@kuleuven.be

Methacryloyl gelatin (GelMA) has gained great attention in the tissue engineering field because of its inherent bioactivity and its tunable physicochemical properties. The fabrication of GelMA constructs by extrusion-based 3D printing is challenging due to the higher elastic behaviour of GelMA inks and clog formation inside the nozzle[1,2]. Herein, we reported the development of a novel cell recognizing, printable and mechanically stable interpenetrating polymer network hydrogel (IPN) based on GelMA and a gelatin-hyaluronan dialdehyde Schiff's polymer as a potential bioink for direct extrusion-based 3D bioprinting[3],[4]. The IPN network was stabilized initially by physical interactions and later by chemical crosslinking of methacryloyl groups in the presence of the radical photoinitiator, lithium phenyl-2,4,6-trimethyl benzoyl phosphinate. IPNs with different concentrations of primary and secondary networks were prepared and analysed for 3D printability and mechanical stability. Temperature sweep rheology measurements show a higher sol-gel transition temperature for IPN hydrogels than GelMA. Moreover, analysing the viability of cells encapsulated in this bioprinted IPN hydrogel construct after day 1, day 3 and day 7 by live/dead imaging followed by semi-quantitative image analysis of live and dead cells by using image J software has shown that the novel material supports cell survival, which makes it an interesting ink for the fabrication of 3D constructs for tissue engineering applications.

Keywords

methacryloyl gelatin; inter penetrating polymer network; hyaluronic acid

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Abstract **#1581**

BIOPRINTING BY LIGHT SHEET LITHOGRAPHY: ENGINEERING COMPLEX TISSUES WITH HIGH RESOLUTION AT HIGH SPEED

Louise Breideband$_P$[1], Francesco Pampaloni[1], Gustaf Mårtensson[2,3], Robert Eklund[2], Helmut Wurst[4], Brigitte Angres[4], Núria Torras[5], Elena Martínez[5], Ruby Shalom-Feuerstein[6]

[1] *Goethe University Frankfurt, BMLS, Physical Biology Group, Germany*

[2] *Mycronic AB, Täby, Sweden*

[3] *Div. NanoBiotechnology, Royal Institute of Technology (KTH), Stockholm, Sweden*

[4] *Cellendes GmbH, Reutlingen, Germany*

[5] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain*

[6] *Department of Genetics & Developmental Biology, The Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology*

Corresponding author's email: balsa@bio.uni-frankfurt.de

Three-dimensional bioprinting (3D bioprinting) has been at the forefront of tissue engineering research in the past years, with ever more efficient systems reaching the market(1). While existing 3D bioprinting techniques are numerous and varied, they are limited by long printing times when used at high resolution(2). The technique described in this work aims at enabling fast and accurate production of monolayered skin constructs.

To achieve shorter production times, a digital scanned light sheet is used to produce patterns of polymerized hydrogel, which enables the printing of a full three-dimensional plane in a matter of a few hundred milliseconds. The high resolution resides in the properties of the light sheet itself – the width of the light sheet represents the z-axial resolution of the system (as low as 10 µm) and the x-axial resolution is determined by the intensity profile of the gaussian beam (around 50 µm). In order to fully exploit this system, the hydrogel used to encapsulate the cells must therefore be tailor-made for photoactivated cross-linking.

As a proof of concept, a light sheet microscope is used as a polymerization source for novel photosensitive hydrogels. The upcoming hardware, software, chemical and biological improvements needed to reach the full potential of this system are expected to eventually be sufficient to print a complete skin construct, which could be used in the drug development industry, or as a graft for regenerative medicine therapy. Additionally, the constructs can be used to reduce and even replace animal testing for drug or cosmetic testing.

Keywords

3D bioprinting; Light sheet microscopy; Stereolithography

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Abstract **#1583**

EFFECT OF MECHANICAL SHAKING DURING CELL-SEEDING PROCESS ON DISTRIBUTION OF CELLS IN DISHES

Koji Ishii[P][1], Takashi Morikura[1], Koki Abe[2], Teiji Sakamoto[2], Takash Kurihara[2], Shogo Miyata[1]

[1] *Keio University*

[2] *Shimizu Corporation*

Corresponding author's email: miyata@mech.keio.ac.jp

Uniform distribution of cells in dish or flask at cell seeding is important for stable cell culture. Especially for expanding culture of pluripotent stem cells (PSCs), the uniform cell distribution is required to maintain pluripotency. During cell-seeding process, cell suspension is placed on dish or flask following mechanical shaking to distribute cells uniformly. However, the effect of mechanical shaking during the cell seeding on the function of PSCs is still unclear. Therefore, the mechanical validation of cell-seeding process is quite important to develop the automated cell culture system for PSCs. In this study, we developed a cell-seeding device to control the rate of cell-suspension injection and mechanical shaking. The cell suspension was loaded on a dish using a syringe pump, and the dish was shaken immediately after cell-suspension loading using motorized stages controlled by computer. Using this cell-seeding device, the effect of shaking speed and amplitude on pluripotency and proliferation of mouse iPSCs was assessed to validate the mechanical process of cell-seeding.

Keywords

iPSC; automated cell culture system; mechanical validation

Abstract **#1585**

SILK FIBROIN REINFORCED DECELLULARIZED CARTILAGE ECM HYBRID SCAFFOLDS FOR ENDOCHONDRAL BONE REGENERATION

Vivek Jeyakumar[P][1], Nedaa Amraish[2], Eugenia Niculescu-Morzsa[1], Christoph Bauer[1], Dieter Pahr[2], Stefan Nehrer[1]

[1] *Center for Regenerative medicine, Danube University Krems, Krems, Austria*

[2] *Department of Anatomy and Biomechanics, Karl Landsteiner University of Health Sciences, Krems, Austria*

Corresponding author's email: vivek.jeyakumar@donau-uni.ac.at

INTRODUCTION:An emerging paradigm in the field of bone tissue engineering is to engineer constructs via the endochondral ossification route. We aimed to fabricate a mechanically stable Silk fibroin (SF) carrier incorporating decellularized cartilage derived extracellular matrix (CD-ECM) and hypertrophic chondrocytes as a model of endochondral ossification and characterize superior bone-like tissue formation nondestructively in the case of in vitro studies.

METHODS: Human bone marrow stem cells (hBMSC's) were seeded onto CD-ECM/SF or SF constructs and primed 2 weeks for chondrogenesis followed by further 6 weeks of hypertrophy priming in differentiation medium. Biochemical assays, SEM/EDX, RT-qPCR, Biomechanical tests followed by µCT scanning were used as methods at 4 and 8 weeks to determine hypertrophy mediated ossification.

RESULTS:Calcium deposition biochemically determined increased significantly from 4-8 weeks in both SF and CD-ECM/SF constructs and retention of sGAG's were observed only in CD-ECM/SF constructs. SEM/EDX revealed calcium and phosphate crystal localization by hBMSC's under all conditions. µCT scanning at 8 weeks indicated a cloud of denser minerals in groups after hypertrophic induction but the BV/TV was higher in CD-ECM/SF constructs than SF constructs. Gene expression by RT-qPCR revealed that hBMSC's expressed hypertrophic markers VEGF, COL10, RUNX2 but the absence of early hypertrophic marker ChM1 and presence of later hypertrophic marker TSBS1 as well as the presence of osteogenic markers ALPL, IBSP, OSX under all conditions.

DISCUSSION & CONCLUSIONS: Incorporation of decellularized cartilage derived ECM onto a mechanical stable silk fibroin scaffold promotes the endochondral ossification route
bone tissue regeneration.

Abstract **#1586**

BIOFABRICATION OF A PERFUSABLE VASCULAR NETWORK SPANNING FROM THE MACRO- TO THE MICRO-SCALE: A KEY FOR TUMOUR MODELS INVESTIGATION

Tristan Bodet[1], Carlos Mota[1], Lorenzo Moroni[1]

[1] *Maastricht University, MERLN Institute. Maastricht, Netherlands*

Corresponding author's email: tristan.bodet@maastrichtuniversity.nl

In the field of regenerative medicine, the lack of vascularization remains a major limitation, while it is essential for the long-term stability of functional tissues. Due to diffusion limitations of oxygen and nutrients up to 300µm from the closest vessel, thick tissues are unable to receive nutrients or eliminate their wastes. This phenomenon leads to tissue necrosis and induces graft rejection. Therefore, to facilitate the connection between the main circulation and the relevant tissue, we aim at developing an artificial vascular network adjusting the vessel's diameter from 1mm to 200µm via a templating technique using extrusion-based additive manufacturing.

The vascular network is produced incorporating a water-soluble carbohydrate glass (Isomalt) inside a fibrin hydrogel. To prevent the template to dissolve prematurely, it was coated with poly(DL-lactide-co-glycolide) (PDLG5002, 50:50; Corbion), to increase its dissolution time. Adapting the coating concentration, we could tune the Isomalt dissolution time and fit with the fibrin hydrogel's crosslinking time. Adjusting the extruding parameters all along the vascular design, we could control the diameter of the produced fibers (from 851µm to 268µm) using a single needle size.

Besides the achievement of fabricating a reproducible hierarchical vascular network, we aim to move from a planar to a free-form design taking advantage of Isomalt fast crystallization rate. This study is a proof-of-concept of the elaboration of a physiologically relevant in vitro tumor tissue model. Moreover, by including cancer cells in the surrounding hydrogel, we aim to investigate the intravasation of metastatic cells into the main circulation toward target tissues.

Abstract **#1587**

REGENERATIVE POTENTIAL OF SPHEROIDS FROM MESENCHYMAL STEM CELLS ON THE MODEL OF MYOCARDIAL INFARCTION

Nastasia V. Kosheleva[1,2], Marina E. Krasina[3], Tatiana V. Lipina[3], Mikhail Yu Karganov[1], Yulia S. Medvedeva[1], Marina A. Lebedeva[1], Sergey I. Rozhkov[4], Irina M. Zurina[1], Irina N. Saburina[1]

[1] *FSBSI "Institute of general pathology and pathophysiology", Moscow, Russia*

[2] *World-Class Research Center "Digital Biodesign and Personalized Healthcare", Sechenov University, Moscow, Russia*

[3] *Lomonosov Moscow State University, Faculty of biology, Moscow, Russia*

[4] *Maxillofacial Hospital for War Veterans, Moscow, Russia*

Corresponding author's email: n_kosheleva@mail.ru

Myocardial infarction occurs after damage and death of cardiomyocytes with replacement be a connective tissue scar, which leads to heart failure. Among the various methods of modeling myocardial infarction in experimental animals, pharmacological model of heart tissue damage using isoproterenol, β1-adrenergic receptor agonist is often used. Mesenchymal stem cells (MSC) are actively used in cell therapy of cardiovascular diseases. In 3D culture in spheroids the proangiogenic and regenerative potential of MSCs is increased. Our aim was to compare the therapeutic potential of MSCs from umbilical cord after their transepicardial injections in the form of cell suspension or spheroids to rats with isoproterenol induced focal myocardial infarction. Injection of MSCs suspension and spheroids reduced number and area of fibrous scars, and inflammatory process in comparison with untreated infarction. Treatment with spheroids was more efficient, by 28 day significantly decreased the number and area of connective tissue scars in left ventricle subendocardium, and ultrastructure of the main cellular organelles was more preserved. Isoproterenol impaired the electrical conductivity of the myocardium manifesting in widening of the QRS complex, lengthening of the QT and QTc intervals, and a decrease in the R wave amplitude. However, in the groups receiving MSCs suspension or spheroids, less severe and prolonged disturbances were recorded in comparison with the group of untreated infarction. Thus, cell therapy with MSCs contributed to normalization of the morphofunctional parameters of the heart. Spheroids exhibited higher efficiency in comparison with cell suspension. This work was financed by the Ministry of Science and Higher Education of the Russian Federation within the framework of state support for the creation and development of World-Class Research Centers "Digital biodesign and personalized healthcare" №075-15-2020-926.

Keywords

multipotent mesenchymal stem cells; spheroids; isoproterenol induced myocardial infarction

Abstract **#1588**

MILD EXERCISE AND MESENCHYMAL STROMAL CELL INJECTIONS ALLEVIATE POST-TRAUMATIC OSTEOARTHRITIS

Jarred Kaiser$_P$[1,2], Jay McKinney[3,1], Fabrice Bernard[3,1], Krishna Pucha[1], Sarvgna Raval[1], Daniel Sok[1], Travis Fulton[1,2], Shannon Anderson[3,1], Brandon Dixon[3], Nick Willett[1,3,2]

[1] *Emory University*

[2] *Atlanta VA Medical Center*

[3] *Georgia Institute of Technology*

Corresponding author's email: jarred.kaiser@emory.edu

Post-traumatic osteoarthritis (PTOA) is characterized by chronic joint inflammation and cartilage degeneration and remains a significant clinical and socioeconomic challenge. Exercise is currently the only clinically proven treatment to provide symptomatic relief and modify disease progression (1). While the exact therapeutic mechanism remains unclear, exercise may resolve chronic inflammation through activation of anti-inflammatory cellular pathways, and by increasing lymph-mediated joint clearance (2). Recent evidence however has revealed that inflammation can lead to dysfunctional mechanotransduction and increased susceptibility to mechanical trauma (3). Thus, concurrent treatment of exercise and injections of mesenchymal stromal cells (MSCs), which are thought to be anti-inflammatory (4), may provide an optimal treatment for PTOA. In these studies, we questioned if, and how, exercise and hMSCs may alter disease progression in a rat model of PTOA. We found that exercise significantly increased the joint clearance rate via the lymphatics, which notably clear both the collagenase sub-group of MMPs and fragmented aggrecan. Exercise also corrected abnormal gait, reduced cartilage attenuation (inversely proportional to sGAG content) and arrested osteophyte growth associated with PTOA. Separately, we found that injection of MSCs significantly reduced pain (secondary allodynia, as measured by von Frey), osteophyte volume, and cartilage surface roughness. We are currently exploring optimal strategies to combine physical and cellular therapies and expect that combined therapy will be more efficacious than either treatment alone. These studies suggest that exercise and MSC injections can restore joint homeostasis, alleviate pain, improve joint function, and attenuate tissue degradation.

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Abstract **#1589**

METABOLIC ALTERATIONS DURING CHONDROGENIC DIFFERENTIATION IN SPHEROIDS: TOWARDS IDENTIFICATION OF METABOLIC MARKERS FOR ENDOCHONDRAL OSSIFICATION.

Niki Loverdou[P][1,2,3], Gabriella Nilsson Hall[4,5], Steve Stengen[6], Kristel Bernaerts[7], Bart Ghesquiere[8], Geert Carmeliet[6], Ioannis Papantoniou[1,4,9], Liesbet Geris[1,10,3]

[1] *Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium*

[2] *Biomechanics Research Unit, GIGA-R In Silico Medicine, Université de Liege, Liège, Belgium*

[3] *Biomechanics Section, KU Leuven, Leuven, Belgium*

[4] *Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium*

[5] *APrometheus, Division of Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium*

[6] *Clinical and Experimental Endocrinology, KU Leuven, Belgium*

[7] *Chemical Reactor Engineering and Safety, KU Leuven, Belgium*

[8] *VIB -KU Leuven Center for Cancer Biology, Belgium*

[9] *Institute of Chemical Engineering Sciences, Foundation for Research and Technology-Hellas (FORTH), Patras, Greece*

[10] *Biomechanics Research Unit, GIGA-R In Silico Medicine, Université de Liege, Liège*

Corresponding author's email: niki.loverdou@kuleuven.be

The use of 3D microtissues following the developmental engineering concept is a promising skeletal tissue engineering strategy 1-3. Considering the important role of metabolism in defining cellular properties4-6 this study aims to identify metabolic quality attributes indicative of a functional cartilage intermediate construct.

We conducted LC-MS (liquid chromatography-mass spectrometry) tracer analysis experiments to gain mechanistic insight during chondrogenic differentiation of osteochondroprogenitors spheroids. 13C labeled glucose and glutamine have been used, as the major energy source for the cells. Our tracer analysis results showed 13C glutamine enrichment in proline from 15% at day 0 to 20 % at day 14 and 42,5 % at day 21 and a similar trend of 13C glutamine contribution to hydroxyproline. Furthermore, we observed progressive 13C glucose enrichment in palmitate from 0% at day 0 to 8% at day 14 and 22% at day 21, suggesting activation of fatty acid synthesis. Subsequently we carried out loss of function experiments by chemically inhibiting fatty synthesis by using the small molecule c75. Results from this study highlighted changes in transcriptome level related to endochondral ossification with significant downregulation of important genes revealing chondrogenic hypertrophy such as IHH, MEF2C, ALP. Additionally, the chemical inhibition of fatty acid synthesis led to poor survival of the in vivo constructs as opposed to the untreated controls where bone organs were formed upon implantation .

This study highlights the possible role of fatty acid synthesis as a metabolic quality attribute of the bioprocess leading to of chondrogenically differentiated engineered microtissues.

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Abstract **#1590**

BIOMIMETIC DESIGN AND FABRICATION OF COMPLEX SKELETAL MUSCLE ARCHITECTURES USING FRESH 3D BIOPRINTING

Maria Stang[P][1], Andrew Lee[2], Brian Coffin[1], Adam Feinberg[1,2]

[1] *Department of Materials Science and Engineering, Carnegie Mellon University*

[2] *Department of Biomedical Engineering, Carnegie Mellon University*

Corresponding author's email: mstang@andrew.cmu.edu

Muscle tissue serves many critical functions including protecting and supporting the skeleton, enabling locomotion, and maintaining homeostasis. Although skeletal muscle has a regenerative capacity, significant impairment or loss of muscle function can result from myopathy and neuromuscular diseases, traumatic injuries, and surgical complications. Unfortunately, current therapies such as functional free muscle transfer and transplantation of exogenous myoblasts are only moderately successful. Engineered skeletal muscle tissue is a potential solution to restore muscle function, but current tissue engineering approaches are not able to recapitulate the various native muscle architectures. Here we report the engineering of skeletal muscle tissues with complex native-like architectures using Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting [1]. Specifically, we engineered highly aligned muscle constructs with parallel, unipennate, and bipennate myofiber architectures using FRESH printed fibrillar collagen scaffolds around which C2C12 myoblasts were cast and compacted in a fibrin gel. Immunofluorescent images demonstrate that the scaffolds provide directional cues to the cells to organize and align along the collagen filaments and fuse into multinucleated myotubes. Various skeletal muscle architectures were created with high fidelity, and the direction and spacing of collagen filaments were shown to be critical to guiding this process. Further, under field stimulation these constructs displayed active muscle contraction and at higher frequencies (20 Hz) approached tetanus with a positive force-frequency relationship. These engineered muscle tissues are the first to demonstrate control of microstructure and myofiber architecture in 3D space and show the potential to recapitulate different muscle types within the body.

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Abstract **#1591**

IN-VITRO MODEL OF THE HUMAN BLOOD VESSEL WALL

Jaroslaw Jacak[P][1], Boris Buchroithner[1], Dmitry Sivun[1], Mario Mayrhofer[1], Markus Axmann[1], Eleni Priglinger[2], Fabian Hauser[1], Sandra Mayr[1]

[1] *University of Applied Scinece, Linz, Austria*

[2] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology (LBI), Vienna, Austria*

Corresponding author's email: jaroslaw.jacak@fh-linz.at

Tissue engineering is a rapidly growing field during the last decade. Cells within an artificial tissue need structural support and guidance for growth. For this purpose, we fabricate polymeric bio-compatible scaffolds by multi-photon lithography (MPL).

In MPL, a femtosecond-pulsed laser focused into a photosensitive resin solution initializes polymerization solely within the focal volume of the laser beam. Hence, sub-micrometer resolution can be achieved in three dimensions. Recently lateral and axial resolution of MPL of below 200nm and around 500 nm have been demonstrated respectively. Hence, its flexible additive manufacturing performance makes MPL a well suited technique for 3D-structuring of biocompatible materials for tissue scaffolds.

The challenge herein is the development of a photoresist that is biocompatible, mechanically stable and can be structured high writing speed. Herein we demonstrate a 2D and 3D biocompatible scaffolds structured onto cell culture membranes, which can be combined with microfluidics. For biocompatibility testing the scaffolds are seeded with cells. In order to promote cell adhesion, we developed strategies to functionalize the scaffolds with biomolecules like antibodies, DNA-linkers or RGD-peptides. This 3D structured cell scaffold within a microfluidic device are seeded with human endothelial cells models of a blood vessel wall. In the future molecular processes like transportation of bio-microparticles or macromolecules will be addressed with our platform.

Abstract **#1592**

INVESTIGATION OF ENDOTHELIAL CELL VIABILITY AND GROWTH ON 3D PRINTED GELMA VASCULAR NETWORKS

Chrisoula Chappell[P][1], Fei Zheng[1], Olga Tsigkou[1], Brian Derby[1]

[1] *The University of Manchester*

Corresponding author's email: chrisoula.chappell@postgrad.manchester.ac.uk

INTRODUCTION: Successful 3D engineered tissues require viable vasculature, however this poses certain challenges [1-3]. As a result artificial channels were formed in a cast photocrosslinked gelatin methacrylate (GelMA) construct by printing sacrificial networks of Pluronic F127. Human umbilical vein endothelial cells (HUVECS) were co-cultured with adipocytes using the above design to produce a 3D viable vascular tissue construct.

METHODS: The one pot synthesis method was used to synthesize Gelatin Methacrylate [2]. Whilst hexagonal shaped pluronic filaments were printed onto glass slides using the robotic I&J 7300-LF printer (Fishnar, UK). Adipose derived stem cells (Thermofisher) were differentiated into adipocytes whilst being embedded within the GelMa structure. The differentiation was assessed using the oil red O stain. Further experimentation looked at the cell-cell interaction and endothelialisation of HUVECS (PromoCell, UK) with the adipocyte model using VE-Cadhering and E Cadhering. Finally the stability of the 3D model was investigated using immediate diffusion at a constant flow rate.

RESULTS: The oil red stain proved the differentiation of the ADSCs into adipocytes. Whilst data on the cell-cell interaction and the endothelialisation will be presented.

DISCUSSION &CONCLUSIONS: Collectively, our data illustrates that ADSCs can be successfully differentiated and they can remain viable after 7 days within the hydrogel matrix.

ACKNOWLEDGEMENTS: CC and FZ were supported by studentships from EPSRC and CSC.

Keywords

Endothelialisation ; Differentiation; Diffusion

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Abstract **#1593**

INFLUENCE OF THE POST-MORTEM INTERVAL ON THE PROPERTIES OF CULTURED HUMAN CORNEAL EPITHELIAL CELLS

Christelle Gross[P][1,2], Gaëtan Le-Bel[1,2], Pascale Desjardins[1,2], Sergio Cortez Ghio[2], Camille Couture[1,2], Lucie Germain[1,2], Sylvain Guérin[1,2]

[1] CUO-Recherche, Médecine Régénératrice, Centre de recherche du CHU de Québec, Hôpital du Saint-Sacrement; Département d'ophtalmologie, Faculté de médecine, Université Laval

[2] Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Médecine Régénératrice, Centre de recherche du CHU de Québec, Hôpital de l'Enfant-Jésus; Département de Chirurgie, Faculté de médecine, Université Laval.

Corresponding author's email: christelle.gross@crchudequebec.ulaval.ca

The tissue-engineered human cornea (hTEC) is a powerful model to study the mechanisms of corneal wound healing and is sparking interest as a suitable substitute for grafting purposes. To ensure hTECs histological and physiological integrity, the primary cultured human corneal epithelial cells (hCECs) must be of the highest possible quality. This study was intended to evaluate the impact of the post-mortem interval (PMI) on the properties of hCECs in vitro. hCECs were isolated from the limbal area of donor corneas either directly upon reception of the tissue (PMI=0) or at different PMI, ranging from 1 to 19 days and grown as monolayers or on hTECs. No noticeable difference in cell morphology was observed. However, short PMI hCECs displayed increased daily doublings and generated more colonies per seeded cell compared with long PMI hCECs. Microarray analyses indicated that short PMI hCECs had a gene expression pattern distinctive from that of long PMI hCECs grown on monolayer. Immunofluorescence analyses revealed that components of the basement membrane and extracellular matrix were deposited similarly in all hTECs, irrespective of the hCECs PMI. Interestingly, wound closure was accelerated in hTECs prepared using short PMI hCECs. Gene profiling analyses on hTECs revealed that most differentially regulated genes encode proteins involved in cell proliferation. Collectively, these results suggest that hTECs reconstructed using short PMI hCECs have a higher number of limbal stem cells and exhibit more efficient wound healing response in vitro, making them the best candidates to produce substitutes for clinical studies.

Keywords

Cornea; Tissue-engineering; Post-mortem interval

Abstract **#1594**

3D PRINTABLE MODELS OF BONE MARROW MECHANICAL ENVIRONMENT

Alexander Regner[P][1], Kalin Gibbons[1], Derek Nesbitt[1], Elizabeth Blaber[2], Trevor Lujan[1], Claire Fitzpatrick[1], Mary C Farach-Carson[3], Danielle Wu[3], Gunes Uzer[1]

[1] *Boise State University, Mechanical and Biomedical Engineering*

[2] *Rensselaer Polytechnic Institute, Biomedical Engineering*

[3] *UTHealth Science Center at Houston, Diagnostic and Biomedical Sciences*

Corresponding author's email: gunesuzer@boisestate.edu

Aged individuals and astronauts experience bone loss despite rigorous physical activity. Bone mechanoresponse is regulated in part by mesenchymal stem cells (MSCs). We reported daily low intensity vibration (LIV) restores MSC proliferation in senescence1 and simulated microgravity2 models, suggesting reduced mechanical signal delivery to MSCs contributes to declining bone mechanoresponse. We developed a 3D bone marrow analog that controls trabecular geometry, marrow mechanics and external stimuli. Finite element (FE) models of hydrogels, representing bone marrow, were generated using instantaneous compression (1000% strain/s, 20% strain) and relaxation experiments (100s) of both gelatin and hyaluronan-based hydrogels. Experimental and in silico vibration experiments using molded-gelatin wells (widths=3, 4, 5, 6 and 8 mm) were performed under 1g acceleration, 100 Hz for FE model calibration. For MSC experiments, 0.25cm3 gyroid-based trabeculae of bone volume fractions (BV/TV)3 corresponding to adult (25%) and aged (13%) mice were printed using polylactic acid. MSCs encapsulated (1x106 cells/mL) in migration-permissive hydrogels4 within printed trabeculae were exposed to LIV (1g, 100 Hz, 1 hour/day). After 14 days, type-I collagen, Ki-67 and f-actin levels (n=3/grp) were quantified for extracellular matrix composition, proliferation, and morphology and grouped with respect to the maximum von Mises strain for 13% and 25% BV/TV scaffolds using the calibrated FE models. As a result of this work, bone analogs will now be flown to the International Space Station in 2023 to determine if consistent mechanical stimulus from LIV counteracts microgravity-induced aging and preserves MSC function, including osteogenesis.

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Abstract **#1595**

SUSTAINED VEGF RELEASE FROM LIVER-SPECIFIC LIGAND-CONJUGATED MICROPARTICLES FOR TARGETED ISLET TRANSPLANTATION

I-Ning Lee$_P$[1], Lisa White[1]

[1] *University of Nottingham Biodiscovery Institute, Division of Regenerative Medicine and Cellular Therapy, School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, UK*

Corresponding author's email: i-ning.lee@nottingham.ac.uk

Islet transplantation is a therapeutical treatment for type 1 diabetes. A major limitation for successful islet transplantation is the failure of graft survival due to difficulty in achieving timely vascularisation. Vascular endothelial growth factor (VEGF) plays a major role in endothelial differentiation and regeneration. Whilst VEGF can be used to promote neo-vasculature, achieving targeted and controlled delivery kinetics remains crucial. One approach is the use of microparticles (MPs) as drug delivery vehicles. However, the major challenge for clinical delivery to liver is that MPs are typically fabricated from commercially available polymers that are non-specific to the organ resulting in inefficient delivery and extravasation.

The asialoglycoprotein receptor (ASGPR) exhibits high affinity as a galactose receptor and is the only liver-specific receptor to be identified thus far. We demonstrated targeted delivery to the liver by synthesising a novel PLGA polymer by covalent conjugation with galactose moieties to provide specific binding sites to hepatocytes in order to enhance MPs retention in liver.

MPs fabricated from this polymer demonstrated a 90% VEGF encapsulation efficiency and exhibited a mean controlled daily release of 0.3 µg of VEGF per 1 mg of MPs between 2 and 7 days of in vitro release suitable to promote vascular development.

We successfully demonstrated a novel synthesis route for galactosylation of commercial PLGA and the fabrication of MPs as a novel protein carrier for targeted liver delivery with high encapsulation and controlled release kinetics of VEGF with enhanced MP retention demonstrated in vivo in mice.

Keywords

microparticles; liver; controlled release

Abstract #1596

CREATING ENVIRONMENTS FOR BONE CO-CULTURES

Sandra Hofmann$_P$[1]

[1] *Eindhoven University of Technology*

Corresponding author's email: s.hofmann@tue.nl

Bioengineering bone aims at growing mimics of bone in the laboratory that could be used either for bone regeneration or as model systems to study bone physiology and pathology. There are commonly three cell types described that are the essential contributors to the bone's native function: osteoblasts, osteocytes and osteoclasts. While all three cell types can be investigated separately, co-cultures of them including their precursors and inactive forms still provide a huge challenge, both in terms of culturing conditions and (quantitative) evaluation of functionality. Cell culture media have been optimized for the requirement of single cell types, supporting tissue development. To simulate bone homeostasis, a physiological microenvironment needs to be created that is supportive of all involved cell types. It should enable the cells to interact with each other in particular through soluble factors. This presentation will elaborate on the current practices of co-culturing bone cells for in vitro bone models. Selected examples will show the high susceptibility of cells towards their biochemical and biophysical microenvironment: for example, that components within fetal bovine serum have the power to mask cell-mediated effects and that they can influence the cellular reaction to mechanical load. Overall, this talk will provide a prospective view on the development of in vitro (co-)culture models as functional representatives of processes occurring in bone.

Abstract **#1597**

GENERATION AND CHARACTERIZATION OF ORGANOTYPIC CARDIAC MICROTISSUES FOR PERSONALIZED MEDICINE

Ece Ergir[P][1,2], Sarah Spitz[2], Jorge Oliver De La Cruz[1], Peter Ertl[2], Giancarlo Forte[1]

[1] Center for Translational Medicine (CTM), St. Anne's University Hospital, International Clinical Research Centre (FNUSA-ICRC), Brno, Czechia

[2] Faculty of Technical Chemistry, Institute of Applied Synthetic Chemistry & Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria

Corresponding author's email: ece.ergir@tuwien.ac.at

As cardiovascular diseases remain the leading cause of death worldwide, there is an increasing focus on developing of physiologically relevant cardiovascular tissue models. The heart, as a chemo-mechano-electrical -biological system, presents special challenges for the establishment of in vitro models suitable for personalized disease modelling and pre-clinical testing of drugs. Although several methodologies to mimic the key features of cardiac complexity exist; current miniaturized in vitro models do not sufficiently address the self-organization and cellular diversity issues at organotypic level. We aim to establish organotypic cardiac microtissues in vitro that contain the different cell types of the heart derived from human induced pluripotent stem cells (hiPSCs), and use this model in an organ-on-a-chip platform to improve microtissue physiology in a precisely controlled dynamic environment.

We have optimized a scaffold-free protocol to generate multicellular, beating and self-organized cardiac spheroids in vitro from hiPSCs, and monitored the self-organization of cardiomyocytes with a fluorescently tagged Cardiac Troponin I reporter. The spheroids result in clusters between 500 microns - 1 mm that gradually self-organize and generate chamber-like structures in long-term culture. They contain multiple cell types of the heart, beat without external stimuli, are viable for more than 50 days, and respond to cardioactive drugs. Furthermore, we have developed a preliminary microfluidic setup that allows long-term term and dynamic cultivation of hiPSC-derived organoids under an interstitial flow regime[1][2][3]. Ongoing prospects include functional characterization of these microtissues in static and dynamic on-chip environments, as well as implementing sensors for continuous non-invasive monitoring of culture parameters.

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Abstract **#1598**

REINFORCEMENT OF ELECTROSPUN PCL NANOMATS WITH HALLOYSITE NANOTUBE AS TISSUE ENGINEERING SCAFFOLDS

Haleh Bakhtkhosh Hagh[P][1], Ali Olad[1]

[1] *Polymer Composite Research Laboratory, Department of Applied Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz 5166614766, Iran*

Corresponding author's email: bakhtkhosh.haleh@gmail.com

In this work, the effect of different concentrations of halloysite nanotubes (HNTs) on the physical, chemical, and biological properties of electrospun polycaprolactone (PCL) nanofibrous scaffold was investigated. HNTs were added to PCL solution at the concentration of 5, 10, 15, 20, and 25 (wt%) regarding PCL matrix, and nanomats were prepared using the electrospinning method. Scaffold samples were studied using FT-IR, XRD, and TGA techniques. The effect of HNTs on the morphology of prepared scaffolds was investigated using SEM imaging. It was found the HNTs with a concentration more than 5% would result in mats with non-homogeneous structure. Also, water contact angel studies showed that higher concentrations of HNTs than 5% had no effect on the hydrophilicity of the scaffold sample. So, scaffold with 5% concentration of HNTs was chosen as the proper scaffold for further investigations. Higher ability of the scaffold with HNTs in comparison to pure PCL sample to induce biomineralization was proved by SEM imaging after 14 days of immersing samples in SBF. MTT assay showed that HNTs would increase the cell viability of fibroblast cells after 24, 48, and 72 h of cell culture.

Keywords

Polycaprolactone; Halloysite; nanocomposite structure

Abstract **#1599**

PROBING THE PH MICROENVIRONMENT OF MESENCHYMAL STROMAL CELL CULTURES ON ADDITIVE-MANUFACTURED SCAFFOLDS

Loretta del Mercato[1], Ivan Lorenzo Molder[2], Anil Chandra[1], Marta Cavo[1], Carlos Mota[2], Dimitrios Kapsokalyvas[3], Giuseppe Gigli[4], Lorenzo Mororni[2], Francesco Colella[P1]

[1] *Nanotechnology Institute of Cnr (NANOTEC-Cnr), Lecce, IT*

[2] *Department of Complex Tissue Regeneration MERLN Institute for Technology-Inspired Regenerative Medicine Maastricht University, NL*

[3] *Department of Molecular Cell Biology Maastricht University Medical Center, Maastricht, NL*

[4] *Department of Mathematics and Physics "Ennio De Giorgi" University of Salento, Lecce, IT*

Corresponding author's email: francesco.colella@nanotec.cnr.it

Despite numerous advances in the field of tissue engineering and regenerative medicine, monitoring the formation of tissue regeneration and its metabolic variations during culture is still a challenge and mostly limited to bulk volumetric assays. Here, a simple method of adding capsules-based optical sensors in cell-seeded 3D scaffolds is presented and the potential of these sensors to monitor the pH changes in space and time during cell growth is demonstrated. It is shown that the pH decreased over time in the 3D scaffolds, with a more prominent decrease at the edges of the scaffolds. Moreover, the pH change is higher in 3D scaffolds compared to monolayered 2D cell cultures. The results suggest that this system, composed by capsulesbased optical sensors and 3D scaffolds with predefined geometry and pore architecture network, can be a suitable platform for monitoring pH variations during 3D cell growth and tissue formation. This is particularly relevant for the investigation of 3D cellular microenvironment alterations occurring both during physiological processes, such as tissue regeneration, and pathological processes, such as cancer evolution.

Keywords

optical sensing; confocal microscopy; 3D in vitro cultures

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Ivan Lorenzo Moldero, Anil Chandra, Marta Cavo, Carlos Mota, Dimitrios Kapsokalyvas, Giuseppe Gigli, Lorenzo Moroni, Loretta L. del Mercato, Small 2020, 2002258

Abstract **#1600**

ADAPTIVE TOOLPATH G-CODING FOR COMPLEX GEOMETRIES PRODUCED BY MELT ELECTROWRITING FOR THICKER AND MECHANICALLY ROBUST BIOENGINEERED MYOCARDIAL CONSTRUCTS

Andrei Hrynevich[P][1,2,3], Madison J. Ainsworth[1,2], Joost van Duijn[1,2,3], Jos Malda[1,2,3], Miguel Castilho[1,2,4]

[1] *Regenerative Medicine Centre Utrecht, University Medical Center Utrecht, Utrecht, the Netherlands*

[2] *Department of Orthopaedics, University Medical Center Utrecht, Utrecht, the Netherlands*

[3] *Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*

[4] *Department of Biomedical Engineering, Technical University of Eindhoven, Eindhoven, the Netherlands*

Corresponding author's email: a.hrynevich@uu.nl

Melt electrowriting (MEW) is an additive manufacturing technology, capable of producing high resolution fibre scaffolds. Recently, MEW scaffolds were suggested as a promising delivery substrate for cardiomyocytes in bioengineered myocardial constructs (BMC)[1]. The scaffold's microarchitecture and mechanical properties have to be precisely adjusted to recapitulate myocardial organization and allow for the scaffold deformation under myocardium contraction.

Several micro architectures, including hexagonal and auxetic[2] shaped, are considered as promising candidates for BMCs. However, an open challenge for MEW is the production of constructs which allow the required local compliant behaviour and anatomical thickness of the infarcted region, without compromising the cardiomyocyte arrangement.

MEW scaffolds with hexagonal pores (0.25-1 mm thick) were printed and analysed. The accuracy of the scaffolds is achieved by minimizing the fibre wall slant, typically observed in MEW scaffolds. This effect was counterbalanced by a layer-by-layer adjustment of parameters (voltage, collector distance and speed) and the printing path. Collector speed and voltage variation significantly weakened the slant effect, however, did not eliminate it. Printing path adjustment showed higher efficiency in fidelity improvement. The adjustment of the stress-strain curve shape of the scaffolds, intended to comply with the different infarction locations, was done by changing the movement path around a pore, while keeping the pore shape and size constant.

The findings of this study push the current thickness and resolution limitations of complex geometry MEW and provide tools for the adjustment of mechanical properties of MEW scaffolds. In combination, these results allow for increased cardiomyocyte delivery in a BMC.

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Abstract **#1601**

3D GLOBULAR SILK FIBROIN/PLURONIC HYDROGELS FOR WOUND HEALING

Azin Khodaei$_P$[1,2], Fatemeh Jahanmard[1], HamidReza Madaah Hosseini[2], Reza Bagheri[2], Narges Johari[3], Saber Amin Yavari[1]

[1] *University Medical Center Utrecht*

[2] *Sharif University of Technology*

[3] *Golpayegan University of Technology*

Corresponding author's email: a.khodaei@umcutrecht.nl

Silk fibroin (SF) hydrogels were used for regeneration of skin wounds due to exudates absorption and their ability to cover the irregular wounds [1]. Recently, various blend hydrogels have been developed to improve the mechanical, structural and biological properties of SF-based hydrogels. Furthermore, many synthetic and natural hydrophilic polymers were used to be blended with SF to accelerate wound healing [2,3]. They were used to improve the SF bio-functionalities, however, their capability to deliver a full depth regeneration is not optimal yet [1,4]. On the other hand, Pluronic is a known FDA approved polymer which significantly reduces the pathogen adhesion and biofilm formation [5]. In this work, we used the amphiphilic structure of pluronic F127 to induce globular nanostructures combined with Curcumin as a conventional antibacterial agent [6] to increase cell penetration.

Extracted SF solution from B. mori cocoons was poured on the NaCl crystals in 1:1 weight ratio. After drying, the salt crystals were washed for several times by MQ-water and the hydrogels were lyophilized. Four experimental groups namely SF, SF-pluronic (SFP), SF-pluronic-acetone (SFP-A), SF-pluronic-acetone-curcumin (SFP-A-Cur) were prepared. The particulate nanostructure of the pore walls showed a diameter of 10 times larger than pure F127 micelles which was due to SF molecules encapsulations [7]. The morphology of attached fibroblast cells was studied through cytoskeleton staining. The confocal micrographs showed that globular microstructure increases cell penetration in the scaffold. The developed platforms could be potentially applied for the full depth regeneration of the skin.

Keywords

Fibroin hydrogel; pluronic; cell penetration

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Abstract **#1602**

CONDUCTIVE HYALURONIC ACID/GELATIN HYDROGELS WITH GOLD NANORODS FOR BIOPRINTING AND TREATING RAT SPINAL CORD INJURY

Emi Kiyotake$_P$[1], Emily Thomas[1], Hannah Homburg[2], Camille Milton[2], Adam Smitherman[3], Nathan Donahue[1], Kar-Ming Fung[4], Stefan Wilhelm[1], Michael Martin[2], Michael Detamore[1]

[1] *Stephenson School of Biomedical Engineering, University of Oklahoma, Norman OK, USA*

[2] *Department of Neurosurgery, University of Oklahoma Health Sciences Center, Oklahoma City OK, USA*

[3] *Department of Neurosurgery, Rush University Medical Center, Chicago IL, USA*

[4] *Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City OK, USA*

Corresponding author's email: emi.kiyotake@ou.edu

A conductive hydrogel comprised of hyaluronic acid (HA), gelatin, and gold nanorods (GNRs) was developed and injected/cross-linked in situ in a thoracic level hemi-section spinal cord injury (SCI) in an 8-week study with 38 rats. The long-term goal is to develop a conductive biomaterial to augment electrical stimulation (ES) for patients with severe spinal cord injury (SCI). In the current work, citrate-GNRs were synthesized with high aspect ratios of 7.7 ± 1.2, as measured from transmission electron microscopy, and were incorporated into HA/gelatin hydrogels at 0.4-1.2 mg/mL concentrations, which were determined by inductively coupled plasma-mass spectrometry. The GNR hydrogel precursors had a bioprintable and paste-like precursor with a yield stress of 511 ± 20 Pa and had a quick crosslinking time (~5 min). The crosslinked hydrogels had conductivities up to 1.15x10^-5 ± 0.19x10^-5 S/cm, and compressive moduli of 83 ± 30 kPa. Seeded rat neural stem cells on GNR hydrogels in vitro had similar adhesion and greater metabolic activity compared to control plated cells. While no significant differences in functional recovery/regeneration were found in vivo from Basso, Beattie, and Bresnahan scoring and immunohistochemistry, we demonstrated the feasibility of applying a paste-like hydrogel precursor in situ for SCI and the safety of the GNR hydrogel. To our knowledge, this was the first study that used a GNR-based conductive hydrogel to treat SCI in vivo, and further provided a translational platform that can be refined to promote axon regeneration and be combined with ES for facilitating functional recovery after SCI.

Abstract **#1603**

CONJUGATION OF REDUCED GRAPHENE OXIDE NANOPARTICLES WITH CTGF FOR DIABETIC WOUNDS AND ULCER TREATMENT

Syed Raza ur Rehman[P][1], Robin Augustine[1], Alap Ali Zahid[1], Anwarul Hasan[1]

[1] *Qatar University*

Corresponding author's email: syedrazaurrahman@gmail.com

Delay in wound healing is a severe problem in a diabatic patient resulting in significant morbidity and mortality, and increased health care costs, caused by prolonged microbial infections, compromised patient's life, limb amputations, and even death. The decrease in blood supply along with oxygen, nutrients, and growth factors leads to severely reduced blood vessel formation (angiogenesis) inadequate cell proliferation and cell migration as well as collagen deposition in wound area [1]. Therefore, there is a dire need for advanced therapeutic modalities that could promote the healing of diabetic wounds by providing a sustained release of oxygen (O2) and growth factors to the wound area and enhance the rapid formation of the blood vessels. Multiple pieces of evidence have shown that CTGF and reduced graphene oxide possesses angiogenic property and enhance wound healing by increasing the oxygen supply to the wound area, and by proliferation and migration of fibroblasts and keratinocytes cells [2]. In this study, we have developed a novel method for the conjugation of CTGF with reduced graphene oxide nanoparticles using EDC-NHS chemistry to accelerate the wound healing process. The rGO/CTGF was then incorporated in GelMA hydrogel to provide a sustained release. The developed hydrogels were then characterized by different physical characterization methods to confirm the morphology of the material. The biocompatibility of the material was confirmed by the cytotoxicity (Live/Dead, MTT) assay using keratinocyte cells. Finally, the wound healing scratch assay shows the ability of the material to promote faster healing of the wound.

Keywords

Wound Healing; Biomaterial; tissue engineering

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Abstract **#1604**

ENGINEERING MINIATURIZED HIGH-THROUGHPUT FREESTANDING MULTILAYERED MEMBRANES FOR BOTTOM-UP TISSUE ENGINEERING STRATEGIES

Elisa Fernandes[P][1], Flávia Lopes[1], Sónia Patrício[1], Clara Correia[1], João Borges[1], João Mano[1]
[1] *Department of Chemistry, CICECO, University of Aveiro, Aveiro, Portugal*
Corresponding author's email: efernandes@ua.pt

Layer-by-layer technology (LbL) has emerged as a powerful strategy for engineering flat and patterned freestanding multilayered membranes to be applied in wound healing, drug delivery and tissue regeneration.[1] However, freestanding membranes still rely on macroscale multilayered systems without spatial control over cell distribution and organization. In this work, miniaturized multi-shaped freestanding membranes were build-up in a multilayer fashion through the alternate deposition of oppositely charged biopolymers onto spatially confined sacrificial hydrogel microarrays. After the LbL assembly process, sacrificial templates were subsequently liquefied releasing dozens to hundreds of individual multilayered micro-membranes with homogeneous size distribution across the different geometries of the underlying templates. The geometrically shaped micro-membranes were cultured with either human adipose-derived stem cells (hASCs) or human umbilical vein endothelial cells (HUVECs) and ultimately assembled into a sandwich-like co-culture aiming to develop a single and complex bone-like 3D microenvironment. The miniaturized cell-seeding multi-shaped membranes hold great promise to be used as microscale modular units for engineering hierarchical heterogeneous biological tissues.

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Keywords
freestanding multilayered membranes; superhydrophobic-superhydrophilic microarrays; modular tissue engineering

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Abstract **#1605**

SUSPENSION BIOPRINTING USING PHOTO-CROSSLINKABLE BIOINKS FOR ENGINEERING ORGANOTYPIC 3D IN VITRO TUMOR MODELS

Vítor M Gaspar[P][1], Maria V Monteiro[1], Marta Rocha[1], Luís P. Ferreira[1], Sónia Patrício[1], João F. Mano[1]

[1] *University of Aveiro*

Corresponding author's email: vm.gaspar@ua.pt

Leveraging additive manufacturing for advancing the fabrication of next-generation in vitro 3D tumor models with personalized hierarchical features, anatomic size and defined morphology is highly advantageous for recapitulating the cellular and extracellular building blocks of the tumor microenvironment from the bottom-up [1]. The development of such biomimetic testing platforms with anatomic length scales unlocks new avenues to program cancer-stromal cells spatial distribution and introduce tumor-ECM components in a user-defined mode that will contribute for accelerating preclinical drug screening and validation. To engineer such organotypic human-sized tumor models, herein we take advantage of freeform suspension 3D bioprinting using continuous viscoelastic supporting baths comprising cost-effective food thickening polysaccharides [2] and tumor-ECM mimetic photo-crosslinkable bioinks laden with key components found in human tumors. This approach enabled the manufacture of shape-defined, cell-rich, 3D multi-layered tumor constructs with high resolution. Malignant and cancer cells remained viable, proliferated within 3D bioprinted ECM-mimetic constructs and secreted key growth factors (e.g. TGF-β, FGF-2). The biofabricated 3D tumor models were highly reproducible and suitable for screening anti-tumoral therapeutics. Overall, the developed approach is highly programable from the bottom-up and transversal, allowing the manufacture of a myriad of anatomically defined 3D tumor testing platforms for screening candidate anti-cancer therapeutics bioperformance in a preclinical in vitro setting.

Keywords

Freeform 3D Biopritning; Tumor Models; Preclinical Drug Screening

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Abstract **#1606**

INNOVATIVE MATERIALS FOR THE PREVENTION OF POST-SURGICAL ADHESION

Franka Ennen-Roth[1], Abdolrahman Omidinia Anarkoli[1], Andreas J. Krüger[1], Laura De Laporte[2,3,1]

[1] *DWI - Leibniz Institute for Interactive Materials, Aachen 52074, Germany*

[2] *Department of Advanced Materials for Biomedicine, Institute of Applied Medical Engineering, RWTH Aachen University, Aachen 52074, Germany*

[3] *Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Aachen 52074, Germany*

Corresponding author's email: ennen@dwi.rwth-aachen.de

The AparTex project focusses on the development of a novel absorbable barrier material to prevent tissue adhesion after surgical interventions.

Adhesion of organs and adjacent tissue after a surgery effects up to 95% of patients during the healing process. This minimizes life quality, can lead to loss of organ function, and displays a huge financial burden on the health care system. Established anti-adhesion materials used as temporary barriers exhibit several drawbacks concerning flexibility, stability, functionality, or handling.

Customized electrospun materials made from functional hydrogel fibers aim to improve the post-operative wound healing and patient recovery.

While one layer of the AparTex textile would stick to the injured tissue, the other layer of the textile will keep the healthy tissue at a distance and act as a physical, absorbable barrier.

Here, we present first prototypes and their properties in terms of mechanical properties, degradation properties and biocompatibility.

Keywords

adhesion; electrospinning; fiber

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Abstract **#1607**

ECM MIMICKING FIBROUS AND VISCOELASTIC SUPRAMOLECULAR HYDROGELS FOR TISSUE ENGINEERING AND 3D PRINTING

Shahzad Hafeez$_P$[1], Antonio Feliciano[1], Agustina Aldana[1], Nicholas M. Matsumoto[2], René P. M. Lafleur[2], Lorenzo Moroni[1], Clemens C.V. Blitterswijk[1], Bert E.W. Meijer[2], Matthew B. Baker[1]

[1] *Department of Complex Tissue Regeneration (CTR), MERLN Institute, Maastricht University, the Netherlands*

[2] *Institute of Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven, the Netherlands*

Corresponding author's email: s.hafeez@maastrichtuniversity.nl

Traditional hydrogel lacks fibrous structure, dynamicity and viscoelasticity found in native extracellular matrix (ECM). ECM is the non-cellular component present within all tissues and consists of fibrous structures like collagen, laminin and fibrin. Nature exploits self-assembly and constituents interact via non-covalent interactions to form superstructures found in ECM. Despite recent advances, creating synthetic analogues of ECM with fibrous structure and controlled viscoelasticity remain a challenge. We have explored the use of 1,3,5-benzenetricarboxamide (BTA) based hydrogelators as synthetic mimics of the fibrous ECM for tissue engineering. First, via modular mixing of BTA, we created supramolecular hydrogels with controlled superstructures and viscoelasticity. BTA hydrogels are highly cytocompatible and can direct cell-cell interactions based on their dynamics and mechanical properties. We then developed a desymmetrization method to upscale and shorten BTA synthesis route. With desymmetrization, molecular control has made possible the development of new fibrous architectures, which are injectable, extrudable and show good shape fidelity after 3D printing. Another challenge is the erosion of highly dynamic BTA hydrogels over time in cell culture. We address this by making BTA monomer with norbornene on it. This newly developed hydrogelator form micron-long fibers. These fibers are polymerized using thiolene chemistry, resulting in tougher and stable hydrogel. This hydrogelator showed high cell viability, very good extrusion and amenable to fabrication via digital light processing. These newly developed hydrogelators opens up numerous applications in tissue engineering, and remain one of the few examples of synthetic and tunable supramolecular hydrogels platforms that show applications in 3D printing.

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Abstract **#1608**

SUCCESSFUL ISOLATION AND CULTURE OF MULTIPOTENTIAL DISTAL AIRWAY STEM CELLS FROM COPD PATIENTS

Tina Patricia Dale$_P$[1], Michael Santer[1], Sana Iftikar[2], Mohammed Haris[2], Nicholas Robert Forsyth[1]

[1] *Keele University*

[2] *Royal Stoke University Hospital*

Corresponding author's email: t.p.dale@keele.ac.uk

Chronic Obstructive Pulmonary Disease (COPD) presents a significant, incurable, worldwide health burden, identifying it as a prospective regenerative medicine target. Sourcing cells for lung therapies is challenging; progress has been made in culturing upper airway basal cells but information on the culture of distal cells remains scarce.

Distal lung from human donors was minced and digested. Cells were plated on type I collagen-coated flasks in cFAD medium with rock inhibitor (cFAD+)[1]. Cells were characterised by immunocytochemistry for vimentin, smooth muscle actin, pan-cytokeratin, TP63, cytokeratin-5, E-cadherin, Club cell secretory protein (CCSP), mucin-5AC and β-IV-tubulin. Differentiated using air-liquid interface culture, with measurement of trans-epithelial electrical resistance (TEER), in a matrigel culture system, and seeded to porous collagen scaffolds.

Culture in cFAD+ resulted in predominantly epithelial cells expressing pan-cytokeratin and E-cadherin, TP63 and cytokeratin-5 suggesting a distal airway stem cell (DASC) identity. At air-liquid interface cells developed tight junctions with increased TEER (≥350 Ω.cm2), increased levels of CCSP, stained positively for β-IV-tubulin and mucin-5AC and had visible, motile cilia. Matrigel culture resulted in self-organising, mucus producing organoids, with visible motile cilia on the surface in addition to thin-walled, cavitated, alveolar-like organoids. The DASCs attached readily to collagen scaffolds, had excellent viability and exhibited proliferation on the scaffolds over a 14 day period.

We have successfully isolated and expanded epithelial progenitors from the distal lung tissue of COPD and healthy donors. Preliminary work demonstrates the DASC multipotential differentiation capacity in a variety of formats making them a valuable source for regenerative medicine therapeutic approaches.

Keywords

Disease models; Distal airway stem cells; COPD

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Abstract **#1610**

HUMAN GROWTH FACTOR FUNCTIONALIZED PHOTO-CLICKABLE HYALURONAN HYDROGELS FOR STEM CELLS 3D CULTURE

Rui J Almeida$_P$[1], Marco Esteves[1], Pedro Lavrador[1], Vítor M Gaspar[1], João F. Mano[1]

[1] *University of Aveiro*

Corresponding author's email: ruijorgealmeida@ua.pt

The biofunctionalization of ECM-mimetic polysaccharide hydrogels with human biomolecule cocktails holds great promise for engineering cell-rich living platforms with improved bioactivity and widespread applicability in numerous biomedical applications. To generate such platforms, herein norbornene-functionalized hyaluronan (HA-Nor) and human-based platelet lysates (hPLs) we combined for engineering click chemistry, orthogonally crosslinkable hydrogel platforms that support mesenchymal stromal/stem cells adhesion and bioactivity. Hyaluronan-norbornene functionalization was performed via a straightforward two-pot grafting-to approach and enabled a rapid combination with hPLs and stem cells under mild conditions, giving rise photo-induced thiol-norbornene crosslinkable networks. Hydrogels fabrication with different concentrations of hPLs impacted constructs viscoelastic properties, swelling kinetics and stem cells metabolic activity. Interestingly, cell viability analysis, via live/dead and metabolic assays, demonstrated that 10% w/v PLs in hydrogels promoted higher cell than their non-functionalized counterparts. Moreover, such platforms were also amenable for MSCs 3D spheroids encapsulation. Overall, the synthesized hybrid platform holds great potential for biomedical applications that can benefit from in situ cells and hPLs delivery, particularly in the context of regenerative medicine.

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Abstract **#1611**

GRAPHENE OXIDE-DOPED GELLAN GUM-PEGDA HYDROGEL MIMICKING THE MECHANICAL AND LUBRICATION PROPERTIES OF ARTICULAR CARTILAGE

Diego Trucco[P][1,2,3], Lorenzo Vannozzi[1,2], Eti Teblum[4,5], Madina Telkhozhayeva[4,5], Gilbert Nessim[4,5], Saverio Affatato[6], Hind Al-Haddad[1,2], Gina Lisignoli[3], Leonardo Ricotti[1,2]

[1] *The BioRobotics Institute, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy*

[2] *Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy*

[3] *IRCSS Istituto Ortopedico Rizzoli, SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Via di Barbiano, 1/10, Bologna 40136, Italy*

[4] *Department of Chemistry, Bar-Ilan University, Ramat Gan 52900, Israel*

[5] *Bar Ilan Institute for Nanotechnology and Advanced Materials (BINA), Bar-Ilan University, Ramat Gan 52900, Israel*

[6] *IRCSS Istituto Ortopedico Rizzoli, Laboratorio Tecnologie Biomediche, Via di Barbiano, 1/10, Bologna 40136, Italy*

Corresponding author's email: d.trucco@santannapisa.it

Introduction

Articular cartilage (AC) is a specialized connective tissue which provides a low-friction gliding surface, supporting shock-absorption and wear-resistance. Nowadays, conventional strategies show several limitations in restoring chondral defects. This work reports the fabrication of a bilayered structure made of gellan gum (GG) and poly(ethylene-glycol) diacrylate (PEGDA), mimicking mechanical and lubrication of AC in deep and superficial zones. Graphene oxide (GO) was analyzed as lubricant agent.

Methods

Blends of GG and PEGDA were crosslinked by UV-light and magnesium chloride. GO was synthesized following modified Hummer's method1, and embedded into the superficial layer. Wear tests, performed following ISO14243, were performed on a knee simulator. Cytotoxic effects on chondrocytes were assessed by Live/Dead and MTT assays.

Results

Mechanical tests allowed to determine the optimal crosslinking parameters, by combining photo (5 min) and ionic crosslinking with MgCl2, to target the Young's modulus of superficial and deep zone2. The presence of GO into the superficial layer provided a lower coefficient of friction in the kinetic regime (~0.03) than the non-doped hydrogels. The wear test confirmed the resistance of the bilayered hydrogel up to 100,000 cycles. The hydrogel formulations did not show any sign of cytotoxicity.

Conclusions

These results are promising in view of the fabrication of a multi-layered synthetic implant for the restoration of AC.

Acknowledgements

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Keywords

Cartilage substitute; Cartilage mechanical properties; Cartilage lubrication properties

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Abstract **#1612**

TOWARDS BIOHYBRID LUNG DEVELOPMENT: DE NOVO SYNTHESIS OF ENDOTHELIAL EXTRACELLULAR MATRIX SUPPORTS MONOLAYER STABILITY UNDER FLOW CONDITIONS

Michael Pflaum[P][1], Katherina Katsirntaki[1], Sophie Jurmann[1], Claudia Hartl[1], Zlata Vukadinovic-Nikolic[1], Axel Haverich[1,2], Bettina Wiegmann[1,2]

[1] *Hannover Medical School*

[2] *German Center for Lung Research*

Corresponding author's email: pflaum.michael@mh-hannover.de

Background

As alternative to lung transplantation, biohybrid lung development, based on the ECMO-strategy, is promoted. Therefore, blood-contacting surfaces, in particular hollow fiber membranes (HFM) will be endothelialized to generate anti-thrombogenic surfaces for long-term application. In order to improve endothelial monolayer stability on HFMs under flow conditions, the impact of extracellular matrix (EM) synthesis, which is crucial to resist dynamic conditions, was assessed during prolonged static cultivation. Furthermore, changes in gene expression of selected EM-proteins due to flow exposure were analyzed.

Methods

EC-monolayers were established on HFMs, incubated for 2 or 6 days under static conditions, and subsequently exposed to flow conditions for 24 hours. For assessing EC-monolayer integrity and EM-synthesis under static and dynamic conditions, immunofluorescence stainings (IF) of VE-Cadherin (CDH5) and collagen-IV (COLIV) were performed. Expression levels of matrix associated genes collagen-alpha-1(IV)chain (COL4A1), syndecan-2 (SDC2), Laminin-alpha4 (LAMA4), hyaluronan-synthase-2 (HAS2), exostosin-1 (EXT1) were analyzed by qRT-PCR. Additionally, cell counting of adherent ECs on HFMs was performed.

Results

Prolonged static cultivation resulted in higher EC numbers on HFM after flow exposure. IF images indicated time-dependend accumulation of COLIV-containing EM. qRT-PCR detected a flow-induced upregulation of the EM-associated genes SDC2, LAMA4, HAS2 and EXT1, while COLIV-associated COL4A1-gene expression was consistent throughout cultivation remaining on the static level.

Conclusion

EM formation is important for long-term stability of the EC monolayer on HFM, especially to resist clinically relevant flow conditions. Therefore, to optimize EM production within HFM endothelialization, further flow adaption protocols need to provide both, time and flow stimuli for EM maturation.

Abstract **#1613**

CD44 EXPRESSION ON INTERVERTEBRAL DISC DEGENERATION: A POSSIBLE THERAPEUTIC TARGET?

Joana Ferreira$_P$[1,2,3], Catarina Meireles[4], Mário Barbosa[5,6,7], Meriem Lamghari[4,6,7], Graça Almeida-Porada[8], Raquel Gonçalves[4,6,7]

[1] *I3S, Instituto de Investigação e Inovação em Saúde*

[2] *INEB, Institute of Biomedical Engineering*

[3] *ICBAS, Institute of Biomedical Sciences, University of Porto, Porto, Portugal*

[4] *I3S*

[5] *I3S*

[6] *INEB*

[7] *ICBAS*

[8] *WFIRM – Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA*

Corresponding author's email: raquelg@ineb.up.pt

Intervertebral Disc (IVD) degeneration is associated with a chronic inflammatory response and extracellular matrix (ECM) degradation. Yet, the cell targets involved in IVD degeneration are still scarcely understood. CD44 is an adhesion receptor highly expressed in Nucleus Pulposus (NP) cells [1], with specificity for several ECM components [2] and reported to be involved in inflammation. Here, we explore the role of CD44 as a possible therapeutic target on IVD degeneration.

Fresh bovine (b)NP were sorted based on CD44 expression and studied for gene expression of inflammatory and ECM remodelling markers. To determine its role in the degenerative cascade, bNP cells CD44 expression was evaluated in IVD organ cultures in healthy vs pro-inflammatory/degenerative conditions and correlated with degenerative IVD mrkers [3]. CD44 distribution on bNP and co-localization with CD14 (monocyte/macrophage marker), was assessed by immunofluorescence (IF).

CD44 is highly expressed on bNP cells (20-40%). CD44+ bNP cells revealed up-regulation of IL-8, but not IL-6 or CD14 gene expression, and significantly down-regulation of aggregan (ACAN), compared with CD44- cells. CD44+ cells also tend to express lower levels of collagen type II (COL2), MMP-2/3, ADAMTS-5 and TIMP2. The pro-inflammatory stimuli significantly increased CD44, alongside CD14, IL-6 and IL-8, while down-regulating COL2 or ACAN gene expression. CD44+ cells co-localized with CD14 in 56±23% cells. These results suggest that CD44+ cells are more associated with the inflammatory cascade, while CD44- cells seem to be more associated with ECM.

Overall, the results show the relevance of CD44 as an important therapeutic target on the degenerative IVD.

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Abstract **#1614**

XENO-FREE CULTURE SYSTEMS FOR HUMAN PLURIPOTENT STEM CELL-CARDIOMYOCYTES DISCOVERED BY HIGH-THROUGHPUT SCREENING.

Aishah Nasir$_P$[1], Laurence Burroughs[2], Jordan Thorpe[3], Grazziela P Figueredo[2], Jan de Boer[4], Chris Denning[3], Morgan R Alexander[5]

[1] *Division of Cancer & Stem Cells, Biodiscovery Institute, University of Nottingham, Nottingham, UK*

[2] *School of Pharmacy, University of Nottingham, UK*

[3] *Division of Cancer & Stem Cells, Biodiscovery Institute, University of Nottingham, Nottingham, UK*

[4] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven*

[5] *School of Pharmacy, University of Nottingham, Nottingham, UK*

Corresponding author's email: aishah.nasir@nottingham.ac.uk

Cardiovascular disease (CVD) remains one of the leading causes of ill-health and mortality. The ability to derive human induced pluripotent stem cells (hiPSCs) from patients and healthy individuals, and then differentiate into cardiomyocytes (hiPSC-CMs) provides new opportunities to model and understand human diseases. Current approaches produce hiPSC-CMs that lack maturity relative to the adult human heart. The use of biomaterials could improve hiPSC-CM functionality by altering biophysical and biomechanical properties including surface chemistry and topography to better represent the in vivo environment.(1)

In this study, high-throughput micro-array screening approaches were used to assess 24,924 cell-polymer interactions(2) and >2000 unique topographies(3). A third platform, ChemoTopoChip: combining polymer and micro-topographies(4) were subsequently used to identify topographically enhanced cell culture systems for improved maturity of hiPSC-CMs. Experiments at scale, focused on assessing hPSC-CM attachment using high-content imaging and functionality by measuring contractility to polymer substrates.

Multi-generation screening of cell-polymer interactions identified high attachment and improved functionality of hiPSC-CMs with amine-containing polymers(5) which also supported attachment of hiPSCs at scale-up. Screening of hiPSCs and hiPSC-CMs with micro-topographies identified how feature size and frequency influences hiPSC pluripotency and expansion as well as structural maturation respectively. Mathematical algorithms identified surfaces that support both hiPSC expansion and differentiations. Topo-chemical combinations are now being tested to investigate the interplay between surface chemistry and topography. The aim being that this will identify xeno-free topographically enhanced culture systems that mediate both hiPSC expansion and in-situ differentiation of hiPSC-CMs with improved maturity and functionality for better CVD modelling.

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Abstract **#1615**

BIOMANUFACTURING PLATFORM DEVELOPMENT FOR THE LARGE-SCALE PRODUCTION OF NON-PARENCHYMAL CELLS TOWARDS THE RECELLULARIZATION OF A PORCINE LIVER

Sara Morini$_P$[1,2], Cristiana Ulpiano[1], Joaquim M.S. Cabral[1], Cláudia Lobato da Silva[1], Pedro M Baptista[2,3,4], Ana Fernandes-Platzgummer[1]

[1] Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

[2] Instituto de Investigación Sanitária de Aragón (IIS Aragón), Zaragoza, Spain.

[3] Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas (CIBERehd), Madrid, Spain. ARAID, Zaragoza, Spain. Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz, Madrid, Spain

[4] Department of Biomedical and Aerospace Engineering, Universidad Carlos III de Madrid, Madrid, Spain

Corresponding author's email: sara.morini@tecnico.ulisboa.pt

Liver transplantation represents the only option for patients with end stage liver disease. Regrettably, this solution is limited by growing demand and inadequate supply of organs available for transplantation. Decellularization is a promising approach in regenerative medicine used to remove the cellular content of organs, preserving a scaffold composed of an intact extracellular matrix and vascular tree. Once decellularized, these scaffolds could be recellularized to create bioengineered organs able to be transplanted.

The main goal of this work is to re-establish a functional vasculature in bioengineered livers of clinical size. Therefore, we are developing a robust reproducible and cost-effective platform for the large-scale expansion of mesenchymal stromal cells (MSCs), smooth muscle cells (SMCs), and endothelial cells (ECs) under dynamic conditions. Firstly, cells were cultured on microcarriers in spinner flasks. Overall, cells reached maximum numbers of 15X106 of MSCs, 20X106 of SMCs, and 7.6X106 of ECs, which corresponds to fold increase values in total cell number of 6, 11 and 3, respectively. Importantly, cells expanded under stirred conditions retained their phenotypical identity. Finally, we were able to expand successfully MSCs and SMCs under fully controlled conditions in stirred tank bioreactors (STBR). Cells reached maximum numbers of 170X106 of MSCs, and 200X106 of SMCs, which corresponds to fold increase values in total cell number of 17 and 33, respectively. As for spinner flask cultures, cells expanded in STBR retained their phenotypical identity. These results could represent an important step toward the production of the necessary amounts of cells required for recellularization purposes.

Abstract **#1617**

DEVELOPMENT OF A NEW BIOACTIVE POLYSACCHARIDE-BASED HYDROGEL FOR GROWTH FACTOR DELIVERY

Marco Sorbona[P][1], Aleixandre Rodrigo-Navarro[1], Manuel Salmeron-Sanchez[1], Cristina Gonzalez-Garcia*[1]

[1] *Centre for the Cellular Microenvironment, University of Glasgow, UK.*

Corresponding author's email: marco.sorbona@glasgow.ac.uk

The rising number of pathologies, including non-union bone defects, skin lesions, neurological disorders and inflammatory processes are greatly debilitating for patients and are becoming an increasing economic burden on the society [1,2]. Most of the clinical remedies result to be palliative and have limited success in alleviating pain and suffering in patients. Regenerative medicine has emerged as a promising field to address these issues. The controlled and targeted delivery of bioactive molecules, such as growth factors (GFs), has shown the potential to induce tissue repair and regeneration [3]. However, despite their efficacy, high doses of GFs are required to obtain significant improvement in clinical settings, leading to severe systemic side effects [4]. Here we present a novel hydrogels system based on acemannan, a bioactive polysaccharide extracted from the inner leaf of the Aloe Vera [5]. The intrinsic anti-inflammatory and immunomodulatory properties make acemannan a promising candidate for tissue regeneration applications. The polysaccharide is engineered to develop 3D hydrogels with tunable physicochemical properties that enable the controlled delivery of GFs, promoting the recruitment and differentiation of stem cells and enabling tissue regeneration. Moreover, the inherent bioactive properties of this carrier, together with the controlled presentation of GFs, will lead to a synergistic effect for efficient tissue repair allowing the use of ultra-low doses of GF, orders of magnitudes lower than the current clinical settings. We believe that this novel hydrogel system will provide a versatile GF delivery platform with potential to be used in different tissue engineering applications.

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Abstract **#1618**

BMP-9 VARIANT WITH INCREASED ECM-BINDING CAPABILITY DISPLAYS SUPERIOR BONE-FORMING CAPACITY TO THOSE OF NATIVE BMP-2 AND BMP-9

Shorouk Fahmy-Garcia[1], Claudia Siverino[2], Nicole Kops[1], Claudio Di Luca[3], Yanto Ridwan[1], Eric Farrell$_P$[1], Gerjo van Osch[1], Joachim Nickel[2]

[1] *Erasmus MC*

[2] *University Hospital Wuerzburg*

[3] *Finceramica*

Corresponding author's email: s.fahmygarcia@erasmusmc.nl

Introduction. BMP-9 has shown stronger osteopromotive capabilities in vitro[1] and higher bone-inducing potential on ridge augmentation[2] than BMP-2. However, intramuscular injection of BMP-9 did not induce bone formation, which might be due to fast diffusion. To avoid this issue, we created a BMP-9 variant (BMP-9v) with ECM-binding sites and then compared its bioactivity in vitro and in vivo to BMP-2 and BMP-9. Methods. ALP expression in C2C12 cells was studied after BMP-9,-2,-9v stimulation. The release profile of BMP-9 and BMP-9v from a collagen scaffold was assessed with ELISA. For in vivo studies 1 and 10 µg of BMP-9,-2,-9v loaded on collagen scaffolds, were implanted subcutaneously in rats (n=8, 6 replicates per condition), scanned biweekly for 8 weeks and retrieved for histology. Results & Discussion. BMP-9 and BMP-9v induced a higher expression of ALP with lower EC50 values compared to BMP-2. BMP-9v was released 10-fold slower than BMP-9 ($p=0.0002$). In vivo, the three proteins induced mineralization at 10 µg with no significant differences. However, 1 µg BMP-9v induced mineralization at similar levels than 10 µg conditions, while 1 µg BMP-2 and BMP-9 barely did ($p\leq0.01$). Furthermore, histological analysis revealed a trabecular-like structure in most of the BMP-9v samples. Indeed, blood vessels and marrow adipose tissue were observed within the bone structure, similarly to the beam-like structures observed in a healthy bone marrow. The promising results of BMP-9v position this new BMP variant as a clinically relevant alternative to current growth-factor based therapies, reducing the supraphysiological doses used to induce bone formation.

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Abstract **#1619**

CHARACTERISATION OF COLLAGEN-BASED SCAFFOLDS AS THREE-DIMENSIONAL GENE DELIVERY MODELS FOR BREAST CANCER TUMOURS AND BONE METASTASIS

Lara Sophie Costard[P][1], Harumi Ramanayake[1], Fergal J. O'Brien[1,2,3], Caroline Curtin[1,2,3]

[1] *Tissue Engineering Research Group (TERG), Department of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland*

[2] *Trinity Centre of Bioengineering (TCBE), Trinity College Dublin (TCD), Dublin, Ireland*

[3] *Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland*

Corresponding author's email: laracostard@rcsi.com

Two dimensional (2D) monolayer cell culture models fail to accurately model the breast cancer (BC) tumour microenvironment and animal models possess numerous limitations. Three-dimensional (3D) collagen-based scaffolds can be utilised to bridge the gap between these models [1,2]. We hypothesise that collagen-based scaffolds, developed in our lab [3], present a more accurate representation of the tumour microenvironment and may be used to assess the potential of nucleic acid delivery to abrogate primary and metastatic BC cell growth. Breast cancer and epithelial cells were grown on collagen-glycosaminoglycan scaffolds to model BC primary tumours and on collagen-nanohydroxyapatite scaffolds to model bone metastasis. Cell behaviour and gene expression comparison was performed comparing cells cultured on 3D scaffolds and in 2D to validate the model. microRNA (miR-146a-5p) delivery was performed to inhibit breast cancer cell growth. Uptake was confirmed by confocal imaging and PCR. DNA and metabolic assays were performed to evaluate effects of miR-146a-5p delivery on proliferation and metabolic activity. Significant changes were identified in gene expression comparing BC cells cultured on the different scaffolds and to 2D. miR-146a-5p was successfully delivered and inhibited BC cell growth in 3D. Differences in the effects of miR-146a manipulation in 2D compared to 3D were demonstrated. This study characterises collagen-based scaffolds as adequate models of BC primary tumours and bone metastasis. The therapeutic effect of nucleic acid delivery correlates with in-vivo effects as evident in the literature [4,5] showing the suitability of these scaffolds to model different BC tumour microenvironments and enable evaluation of gene therapy.

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Abstract #1620

EFFECT OF NOZZLE SIZE AND PRINTING SPEED ON THE PRINTABILITY OF A HYBRID DECM/POLYSACCHARIDE BIOINK

Leonardo O.P. Longo[P][1], Renata F Bombaldi de Souza[1], Fernanda C Bombaldi de Souza[1], Emerson G Moretto[1], Tácia T.A. Liguori[1], Gabriel R Liguori[1]

[1] *TissueLabs SAGL*

Corresponding author's email: gabriel@tissuelabs.com

INTRODUCTION: One of the elementary issues with 3D bioprinting is the proper set-up of the printing parameters to achieve well-defined geometries. Among them, the nozzle size and printing speed are particularly critical. Herein, we investigated the effect of these two parameters on the printability of a hybrid decellularized extracellular matrix (dECM)/polysaccharide bioink. METHODS: Alginate-xanthan solution (1:3) was mixed with a lung dECM hydrogel at a 90:10 weight ratio. Four nozzle sizes (18G, 20G, 22G, and 25G) and printing speeds (0.5mm/s, 1mm/s, 2mm/s, and 4mm/s) were tested at a standardized flow rate. Printability was assessed by filament fusion test using a piston-based 3D bioprinting system. RESULTS: Although all the combinations of nozzles and printing speeds allowed the bioprinting of our bioink, printability differed among the distinct setups. The percentage of defective lines was 1.7%, 10.0%, 30.0%, and 51.7% for increasing nozzle sizes. Defective lines were 31.7% at 4mm/s and around 20% for the other speeds. The spreading of the bioink (diffusion rate, DR) was higher than 60% for all printing speeds and pore sizes for 18G. For 25G, the DR remained between 20% and 60% for all speeds and pore sizes above 2mm. DR for 20G was higher than 22G for all speeds for pores above 2mm. CONCLUSION: The use of a 22G nozzle with a printing speed of 1mm/s or less resulted in the best printability for our hybrid dECM/polysaccharide bioink.

Keywords

Bioprinting parameters; Nozzle size; Printing speed

Abstract #1621

MULTISCALE 3D MICROPHYSIOLOGICAL SYSTEMS TO SCREEN JOINT THERAPEUTICS AND REGENERATIVE APPROACHES

Riccardo Gottardi[1,2,3]
[1] *University of Pennsylvania*
2 Children's Hospital of Philadelphia
3 Fondazione Ri.MED
Corresponding author's email: ricgottardi@gmail.com

Tissue-tissue interactions, such as those between cartilage and bone in the osteochondral junction, cartilage and ligament in the enthesis, bone and vasculature, etc. play a critical role in joint diseases and repair. However, it is challenging to study tissue interfaces in vitro since each tissue requires a defined and often very different microenvironment. We developed a multiscale approach that combines microfluidic and mesofluidic bioreactor systems that maintain media separation for in vitro culture and engineering of OC tissues and constructs of progressively greater complexity. With this approach, it is possible to perform progressive steps of screening from organoids in a microfluidic system, to constructs that match the dimensions and are closer to the complexity of native tissues, to actual composite human tissue such as the osteochondral unit. Furthermore, this platform preserves the possibility of interrogate tissue-tissue crosstalk thus assessing drug interactions. Importantly, the platform enables mechanistic studies pathological processes and to test the effectiveness of regenerative and tissue engineering approaches, including novel scaffold technologies, prior to application in vivo.

Abstract **#1623**

COUPLING ANGIOGENESIS AND OSTEOGENESIS FOR ENHANCED IN VIVO BONE REPAIR USING SCAFFOLDS AND COMBINATORIAL MICRORNA THERAPY

Caroline M Curtin[P][1,2,3], Irene Mencía Castaño[1], Rosanne M Raftery[1], Gang Chen[4], Brenton Cavanagh[5], Brian Quinn[1], Garry P Duffy[6], Fergal J O'Brien[1,2,3]

[1] *Tissue Engineering Research Group, Royal College of Surgeons in Ireland, Dublin, Ireland*

[2] *Trinity Centre of Bioengineering (TCBE), Trinity College Dublin (TCD), Dublin, Ireland*

[3] *Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI & TCD, Dublin, Ireland*

[4] *Department of Physiology and Medical Physics, Centre for the Study of Neurological Disorders, Microsurgical Research and Training Facility, RCSI, Dublin 2, Ireland*

[5] *Cellular and Molecular Imaging Core, RCSI, Dublin 2, Ireland*

[6] *Department of Anatomy, School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, University Road, Galway, Ireland*

Corresponding author's email: carolinecurtin@rcsi.ie

Coupling angiogenesis and osteogenesis within bioactive tissue engineered scaffolds represents a promising approach to recapitulate the natural bone repair process. Several miRNAs have shown potential to influence the angiogenic and osteogenic capacity of mesenchymal stem cells (MSCs) (1). Interestingly, miR-210 and miR-16 possess counter-active targets involved in both angiogenesis and osteogenesis: miR-210 acts as an activator by silencing EFNA3 & AcvR1b, while miR-16 inhibits both pathways by silencing VEGF & Smad5. We hypothesized that dual delivery of miR-210 mimic and miR-16 inhibitor (antagomiR) from a collagen-nanohydroxyapatite scaffold system developed in the lab (2, 3), may hold potential for bone repair, and assessed its potential to accelerate bone repair by directing enhanced angiogenic-osteogenic coupling in host cells in a rat calvarial defect. The nanomiR-210 mimic group significantly increased miR-210 levels and silenced AcvR1b and EFNA3, while nanoantagomiR-16 treatment resulted in >90% silencing of miR-16 levels, followed by enhanced Smad5 and VEGF expression. Silencing of direct targets was remarkably improved with the nanomiR-210/16 dual treatment. Both nanomiR-210 mimic and nanoantagomiR-16 enhanced VEGF secretion and calcium deposition by hMSCs, with levels further increased in the nanomiR-210/16 dual group. These dual-miRNA loaded scaffolds showed more than double bone volume and vessel recruitment increased 2.3-fold in the defect site over the miRNA-free scaffolds only 4 weeks after cell-free implantation in vivo. This work represents a highly promising approach to recapitulate the natural bone repair process in a 3D scaffold miRNA delivery system and underlines the potential of these scaffolds as 'off-the-shelf' miRNA-therapeutic platforms.

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Abstract **#1624**

FIBROBLAST DERIVED MATRIX SKIN EQUIVALENT, FDMSE, A TOOL TO STUDY CONTINUES ENVIRONMENTAL AND DRUG RELATED IMPACT.

Elizabeth Pavez Loriè[P][1], Katharina Janke[1], Philipp Worst[1], Petra Boukamp[1,2]

[1] *Leibniz Research Institute for Environmental medicine*

[2] *DKFZ German Cancer Center*

Corresponding author's email: elizabeth.pavezlorie@iuf-duesseldorf.de

To understand the development of disease or the impact of the environment, we need to use in vitro models that can endure a more realistic exposure of not only a few days, but several weeks or months. Our scaffold free and self-assembled dermal compartment made of normal human fibroblasts (fdmDE) achieves an in vivo-like dermis that can sustain epidermal regeneration for several months, reaching tissue epidermal homeostasis, building a stable and reproducible skin equivalent (fdmSE) (1). The versatility of the model not only mimics normal human skin, but also different degrees of malignancy by using different transformed cells (like HaCaT and HasKpw cells) as well as photoaging phenotypes (using fibroblasts from sun exposed skin of old donors). With this tool we have studied the effect of experimental sunlight (simultaneous combination of UV, visual and infra-red radiation) comparing it to UV exposure in young and old normal human skin equivalents as well as HaCaT-based models. In addition, and due to the fdmSE's lack of immune cells, we studied the immune suppression-independent modalities of the immunosuppressant, cyclosporin A (CsA), which increases the risk for cutaneous squamous cell carcinomas (cSCC). The facility of extended exposure of these models has been key to understand their sustained impact on tissues-organ interplay, such as the loss or gain of certain extra cellular matrix proteins, the increase of ECM degradation or the changes in epidermal tissue behaviour. Therefore, we suggest the use of these models to include the important dimension of time into skin in vitro modelling.

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Abstract **#1625**

HYBRID HYDROGEL/3D-PRINTED SCAFFOLD COMPOSITES IN COMPLEX GEOMETRIES WITH TUNABLE SMALL MOLECULE RELEASE

Nettie E Brown[P][1], Scott J Hollister[1,2], Johnna S Temenoff[1,2]

[1] *Wallace H. Coulter Department of Biomedical Engineering, Georgia Tech*

[2] *Petit Institute for Bioengineering & Bioscience, Georgia Tech*

Corresponding author's email: nettie.brown@gatech.edu

Temporomandibular joint (TMJ) degeneration affects over 10 million Americans. Tissue-engineered TMJ reconstruction will require scaffolds that provide mechanical support and provide controlled delivery of biological factors to induce tissue regeneration(1). To address both needs, the goal of this study was to control the release of a model small heparin-binding molecule, crystal violet(CV), through a tunable PEGDA/heparin hydrogel layered within a porous 3D-printed load-bearing scaffold. Laser-sintered porous PCL scaffolds were placed in custom molds and injected with hydrogel solution prior to 20-minute thermal crosslinking at 25°C. Phase-contrast microscopy demonstrated hydrogel penetration throughout void spaces of the 3D-printed porous scaffold in two distinct layers representing cartilage and bone compartments. MicroCT 3D reconstruction revealed ~100% gel uptake in porous scaffolds compared to the theoretical volume from scaffold design software. Stress-relaxation compression tests demonstrated viscoelastic properties for composites(~23% decrease in force after 5-minute constant strain). Scaffolds were modeled as a homogenized cylinder with effective hyperelastic properties and optimized to experimental data to determine material coefficient(c1) in the Neo-Hookean hyperelastic material model: $W = C1(I1-3)$. C1 was 1.24 MPa with an R2 of 0.99, verifying that the composites can be modeled as a nonlinear elastic Neo-Hookean material during the ramp-up phase of viscoelastic testing. Zero-order release of CV was observed over 7 days. Cumulative release decreased by ~24% when heparin wt.% was increased from 10 to 15 due to greater electrostatic interactions with the positively-charged CV. Further development of our composite system may lead to an alternative to current treatments for anatomically complex osteochondral defects.

Keywords

3D Printing; Hydrogels; Scaffolds

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Abstract **#1626**

MODIFICATION OF NATURAL ECM GELS WITH TRIFUNCTIONAL OLIGOURETHANES/SILICA PARTICLES AND ITS IMPLICATION IN THE PROMOTION OF AN ANTI-INFLAMMATORY MICROENVIRONMENT

Paola García[P][1], Pedro Ulises Munoz-Gonzalez[1], Laura Castellano[1], Birzabith Mendoza[1]
[1] *División de Ciencias de Ingeniería; Universidad de Guanajuato, León, México.*
Corresponding author's email: ep.garciatejada@ugto.mx

The modification of extracellular-matrix (ECM) gels with trifunctional-oligourethane(TO) and silica is proposed to generate templates for the treatment of pathologies with a highly inflammatory environment such as the degenerative intervertebral discs (DID). The role of the crosslinkers derived from lysine diisocyanate and ethoxylated glycerol, and modifiers of silica particles is evaluated to generate an anti-inflammatory response in the context of DID. Gels were manufactured by crosslinking of ECM materials (pericardial tissue or Achilles tendon) with trifunctionalized-oligourethanes (CX) and optionally doped with silica (CXSi). The gelation kinetics(turbidimetry/rheometry) of the crosslinked hydrogel indicated a gelation in 40min at 37°C. The crosslinking degree, estimated by quantification of residual primary amine groups, was of 27% for CX and 31% for CXSi. The diameter of the formed fibers in the gels was estimated by turbidimetry in 72.8nm, with a mass-length ratio of 2.39x10^12(Da/cm). The G' of the gels was around 50–80Pa transitioning to a stable-gel capable of in-situ after injection. The strain required to make the ECM gel flow correlates with the increase in the type I collagen concentration; however, the required strain increases for the crosslinked gels. The injection of the CXSi gels induced a significant reduction in the expression of genes that code the pro-inflammatory markers IL-6 and iNOS in an IL-1β-induced inflammation ex vivo model in bovine intervertebral discs. In contrast, the CXSi gel application upregulated the gene expression of aggrecan. Further studies are underway to evaluate the performance of the CXSi gels in promoting an anti-inflammatory microenvironment in the DID context.

Keywords
DEGENERATIVE INTERVERTEBRAL DISCS; CROSSLINKED HYDROGELS DOPED WITH SILICA; INJECTABLE COLLAGEN HYDROGELS

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Abstract **#1627**

IN VIVO SIMULATIVE BIOCOMPATIBILITY ASSESSMENT OF BIORESORBABLE CARDIOVASCULAR SCAFFOLDS

Jochen Salber$_P$[1], Siegfried Shah[1]

[1] *Salber Laboratory, Centre for Clinical Research, Department of Experimental Surgery, Ruhr-Universität Bochum, Bochum, Germany*

Corresponding author's email: jochen.salber@ruhr-uni-bochum.de

PCI is a very successful method of placing stents in occluded coronary arteries. This restores blood flow in patients suffering from acute or sub-acute coronary occlusion. Research continues to be conducted worldwide on new biocompatible materials for use in stent devices, which on the one hand offer sufficient strength for vascular support during constructive remodeling and on the other hand allow good cyto- and hemocompatibility. Complications continue to occur with stent implantation; the main problems are in-stent restenosis and thrombosis due to inadequate mechanical properties or poor biocompatibility. A major drawback of BMS and DES is the durability of the stent scaffold, which is associated with several adverse events. Fully resorbable stents are the latest development. It is believed that scaffold resorption following vascular repair offers several benefits, including reduced risk of late stent thrombosis, avoidance of repeat treatments and prevention of lateral branch obstruction. To guarantee highly valid and reliable preclinical data, countless animal experiments have to be carried out. This is unethical, costs a lot of money and the results are often not completely transferable. To address this, we have developed a biomechanoreactor using ex vivo porcine heart coronary arteries obtained from regular pig slaughter to study novel and conventional stents under blood flow conditions in direct comparison regarding their biocompatibility over time. The data on biomechanics, degradability, drug release and reendothelialization of different stent types collected over 12 weeks will be used for in silico simulations. In this way, animal experiments can currently be reduced by 50%.

Abstract **#1628**

SPRI-BASED BIOSENSOR FOR THE DETECTION OF CIRCULATING EXTRACELLULAR VESICLES AND THE MONITORING OF REGENERATIVE REHABILITATION EFFICACY

Marzia Bedoni[1], Alice Gualerzi[1], Silvia Picciolini[1], Arianna Iannone[1], Cristiano Carlomagno[1], Angelo Montesano[1]
[1] *IRCCS Fondazione Don Carlo Gnocchi, via Capecelatro 66, Milan, Italy*
Corresponding author's email: agualerzi@dongnocchi.it

The combination of rehabilitation protocols and regenerative therapies offers the outstanding opportunity to promote and enhance the endogenous regenerative and repair processes occurring in tissues damaged or lost due to injury, disease, or age. Still, one of the main hurdles in the clinical approach to regenerative rehabilitation is the lack of easily accessible and sensitive biomarkers for the evaluation of rehabilitation and therapy efficacy. Extracellular vesicles (EVs) are nanoscaled vesicles that mediate intercellular communication among organs. EVs were shown to be involved in the onset, progression and resolution of many disorders, being also used as valuable tool in the regenerative medicine field. However, the initial enthusiastic approach to EVs has been hindered in its transfer to clinics because of technological obstacles related to their dimensions and to their limited amount. We show herein the application of a Surface Plasmon Resonance imaging (SPRi)-based biosensor(1) for the detection and characterization of blood EVs from stroke patients, before and after rehabilitation. After the successful SPRi detection of EVs of different cellular origin (brain and non-brain cells), the quantification of specific surface molecules related to pathological or regeneration processes was accomplished. Our results demonstrated the ability of the SPRi biosensor to reveal differences in the relative amount of specific cell-derived EV subpopulations and in their cargo during disease progression and rehabilitation induced recovery. Our results provide support for using the proposed SPRi-based biosensor for the detection and characterization of circulating EVs in order to evaluate the efficacy of rehabilitation protocols and regenerative therapies.

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Abstract **#1630**

ETHICAL CONSIDERATIONS FOR RESPONSIBLE RESEARCH AND CLINICAL DEVELOPMENT OF CHIMERIC BIOARTIFICIAL PANCREASES

Dide de Jongh[P][1,2], Eline M. Bunnik[3], Emma K. Massey[4]

[1] *Department of Medical Ethics and Philosophy of Medicine, Erasmus MC, University Medical Centre Rotterdam, The Netherlands.*

[2] *Department of Nephrology and Transplantation, Erasmus MC, University Medical Centre Rotterdam, The Netherlands.*

[3] *Department of Medical Ethics and Philosophy of Medicine, Erasmus MC, University Medical Centre Rotterdam, The Netherlands*

[4] *Department of Nephrology and Transplantation, Erasmus MC, University Medical Centre Rotterdam, The Netherlands*

Corresponding author's email: D.dejongh.1@erasmusmc.nl

The most effective treatment for type 1 diabetic patients is transplanting a whole pancreas or islet cells. However, major limitations of allogeneic transplantation are organ shortage and the need of life-long immune suppression medication. In the last decade, the field of regenerative medicine has combined engineering and biological techniques in the attempt to regenerate organs. The European VANGUARD project, funded by the European Commission, aims to engineer immune-protected bioartificial pancreases for transplantation into non-immunosuppressed type 1 diabetic patients. This project is creating a 'combination product' using cells from a variety of sources, including animals, donors and patients, resulting in a chimeric bioartificial organ for transplantation. Developing such a product faces several research-ethical issues. One pertains to the risks and burdens of first-in-human clinical trials, and subject selection. Under what conditions can the product safely be tested in humans for the first time? Which research participant group should be invited first for participating in clinical trials to receive a transplantable bioartificial pancreas? The aim of our study, which is part of and runs in parallel with the VANGUARD project, is to investigate the ethical conditions for responsible research when bio-engineered pancreatic organs are developed and tested in clinical trials, e.g. subject selection, study design, informed consent and risk-benefit assessment, and contribute to research-ethical guidance in the field of regenerative medicine. In this presentation, we present the results of a review of the literature on the ethics of regenerative medicine, and discuss one or two issues more in depth.

Abstract **#1631**

GRAPHENE OXIDE COATED DECELLULARIZED ARTERIES WITH IMPROVED MECHANICAL AND BIOLOGICAL PERFORMANCE

Andreia Trindade Pereira[P][1], Karl H. Schneider[2], Patricia Henriques[3], Sofia Melo[3], M. Cristina L. Martins[4], Helga Bergmeister[2], I. C. Gonçalves[5]

[1] *INEB - Instituto de Engenharia Biomédica Abel Salazar*

[2] *Center for Medical Physics and Biomedical Engineering, Medical Univ. of Vienna, Austria*

[3] *INEB/i3S - Instituto de Engenharia Biomédica, Portuga*

[4] *INEB/i3S - Instituto de Engenharia Biomédica, Portugal*

[5] *INEB/i3S - Instituto de Engenharia Biomédica, Portugal;*

Corresponding author's email: andreia.pereira@i3s.up.pt

Coronary artery disease affects 335M people worldwide. There are no small-diameter vascular grafts to compete in the replacement of coronary arteries with autologous grafts, often unavailable. Decellularized arteries (dArteries) emerge as preeminent alternative, but have unsatisfactory mechanical and biological performance, due to thrombosis and infection. This study proposes the coating of dArteries with graphene oxide (GO) to surpass these limitations.

Human placenta/umbilical cord arteries were isolated and decellularized [1], exhibiting low amounts of DNA. Two different GO were purchased or produced by modified Hummers' method [2] and perfused through the dArteries lumen to coat it. XPS analysis showed that both GO present similar atomic composition, ~34%O/66%C, while DLS and TEM imagens showed that they have different sizes, 1.3µm(sGO) and 1.9µm(lGO). GO-coatings are homogeneous, stable and completely cover the inner lumen of the dArteries, as shown in SEM images. Uniaxial tensile tests of dArteries rings showed that only the lGO-coating of umbilical cord dArteries increases 29% burst pressure and 25% strain, reaching values similar to human saphenous vein. In vitro biological assays showed that GO coatings reduce human platelets and bacteria (S. aureus) adhesion to dArteries without compromising endothelial cells (HUVECs) adhesion. Preliminary in vivo acute thrombogenicity studies (porcine AV-shunt) show that heparin covalent binding to both uncoated and GO coated dArteries seems to prevent their occlusion when exposed to non-heparinized blood.

Overall, lGO-coatings have a significant role in improving dArteries performance, representing a step forward in the transition from lab bench to market of these grafts.

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Abstract **#1632**

ANGIOGENIC AND CELL PROLIFERATIVE MICRONEEDLE ARRAY FOR RAPID HEALING OF CHRONIC WOUNDS

Anwarul Hasan$_P$[1,2], Abdulla Al Mamun[1,2], Rashid Ahmed[1,2], Robin Augustine[1,2]

[1] *Department of Mechanical and Industrial Engineering, College of Engineering, Qatar University– 2713, Doha, Qatar*

[2] *Biomedical Research Center, Qatar University– 2713, Doha, Qatar*

Corresponding author's email: hasan.anwarul.mit@gmail.com

Non-healing wounds are among serious complications of diabetes resulting in significant morbidity and mortality, and increased health care costs, caused by prolonged microbial infections, limb amputations, and even death. Therefore, there is a dire need for advanced therapeutic modalities that could promote diabetic wound healing. One of the major reasons for the delayed healing of diabetic wounds is poor supply of oxygen (O2) that causes decreases in cell viability, cell migration and cell proliferation. Lack of vascularization is another challenge in diabetic wound healing. A number of potential solutions have been investigated to solve the problem of delayed healing of diabetic wounds including the use of growth factors, stem cells, and combinations of both . However, the high cost, fast degradation, rejection of cells by the immune system restrict their effective use. The short shelf life and low stability of growth factors during the transportation and storage conditions as well as their high costs pose a huge barrier towards the commercial application of these growth factors-based formulations for widespread clinical use. Studies indicated that exogeneous delivery of nitric oxide (NO) can promote angiogenesis. Herein, we developed a microneedle array-based patch for diabetic wound healing that can effectively deliver a sustained amount of O2 and NO through the micro sized needles to achieve an angiogenic effect and abundant supply of O2. We noticed that the delivery of NO and O2 using microneedle array-based patch promoted new blood vessel formation around wound sites and hence increased the healing of refractory diabetic wounds.

Keywords

Microneedle array; Oxygen releasing; angiogenesis

Abstract **#1633**

HIGH-THROUGHPUT GENERATION OF HUMAN BONE MARROW CELL SPHEROIDS IN MICRODROPLETS FOR CARTILAGE TISSUE ENGINEERING

Juan Aviles Milan[P][1,2], Jonathan Dawson[1], Jonathan West[2], Rahul Tare[1], Xize Niu[2]

[1] *Bone and Joint Research Group, Centre for Human Development, Stem Cells & Regeneration, University of Southampton, Southampton, SO16 6YD, UK*

[2] *Engineering and the Environment and Institute for Life Sciences, University of Southampton, Southampton SO17 1BJ, UK*

Corresponding author's email: jam1e16@soton.ac.uk

Osteoarthritis affects a large proportion of the aged population worldwide. Cartilage tissue engineering approaches have emerged to replace invasive surgical techniques aiming to restore articular cartilage [1]. Human bone marrow stromal cells (HBMSCs) offer the potential as a cell source for differentiation into cartilage. However, their application is hindered by a crucial limitation: subpopulation heterogeneity [2].

The aim of this study was to achieve high-throughput cell aggregation to test the hypothesis that SOX9 was an early marker upregulated upon condensation and could be used to enrich HBMSC chondroprogenitor subpopulations.

HBMSCs were injected into a PDMS chip with continuous gentle stirring. QX200™ generation oil (Bio-Rad) was used for droplet stabilisation. Droplets were incubated at 37°C, 20% O2 and 5% CO2 in chondrogenic differentiation medium. Aggregates were later recovered from microdroplets on days 1, 2 and 3 for biological characterisation (i.e. colony formation assay, RT-qPCR and immunofluorescence).

The droplet microenvironment enabled the formation of minimal HBMSC aggregates (comprising only a few cells) within one hour of culture. HBMSCs aggregates displayed maintained colony formation functionality when re-plated for 14 days. In addition, HBMSC aggregates expressed higher SOX9 mRNA levels than monolayer cultures as early as one day of chondrogenic induction. At that time point, aggregates expressed SOX9 at the protein level with marked heterogeneity among them.

The work presented in this study confirms the potential of application of droplet microfluidics for cell aggregation, short-term culture and sorting on the basis of early markers opens new prospects for harnessing HBMSCs for cartilage tissue engineering.

Keywords

Droplet ; Microfluidics; Spheroids

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Abstract **#1635**

CHITOSAN-BASED NANOFIBROUS WOUND DRESSING WITH ALTERED ANTIBACTERIAL ACTIVITY

Miroslava Rysová[P,1], Hana Tománková[1], Marcela Munzarová[2], Tomáš Zajíc[3,1], Alena Ševců[1]

[1] *Technical University of Liberec, Institute for Nanomaterials, Advanced Technology and Innovation, Liberec, CZ*

[2] *Nano Medical Inc., Liberec, CZ*

[3] *Regional Hospital, Centre for Laboratory Medicine, Liberec, CZ*

Corresponding author's email: miroslava.rysova@tul.cz

Wound management, nutrition and drug delivery do constantly attract significant attention leading to recent evolution of nano-based dosage forms and wound dressings for active wound management of contaminated and slow-to-heal wounds. Among all types of nanomaterials, nanofibres have shone due to their unique properties leading to barrier effect and increased drug bioavailability. In addition to these properties held by nanofibres in general, the chemical nature is a crucial parameter, possibly able to modulate wound healing process through molecules released into the wound site. From this point of view, chitosan-based nanofibres represent a very promising material based on natural and sustainable material, which can be manufacture using green solvents and doped with multiple active compounds enhancing their natural antibacterial activity. In this study, we evaluated impact of Zn+, Ag+ and Cu2+ ions as potential bioactive dopants. Ion incorporation into electrospun chitosan nanofibres was studied in terms of effects on bacterial film elimination (E. coli and S. gallinarum), cytocompatibility to normal human dermal fibroblasts (NHDF) and Hacat keratinocytes, cellular stress and proliferation and pro-inflammatory cytokines (IL-1α and IL-6) expression. The bioactive ions doped chitosan nanofibres exhibited altered antibacterial activity against both bacterial strains in comparison to pristine chitosan nanofibres. The strongest antibacterial effect was observed in the case Ag+, yet incorporation of Zn+ and Cu2+ led to higher cell viability and modulated cellular stress in comparison to Ag+. This work was supported by projects HyHi (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000843) and TAČR FW17134.

Abstract **#1636**

ENGINEERED NEURAL TISSUE FABRICATED FROM DECELLULARISED ECM HYDROGELS FOR PERIPHERAL NERVE REPAIR

Simon C. Kellaway[P][1,2], Victoria Roberton[1], James B. Phillips[1], Lisa White[2]

[1] *University College London*

[2] *University of Nottingham*

Corresponding author's email: ucnvsck@ucl.ac.uk

Introduction

Engineered neural tissue (EngNT), an artificial tissue made using purified type I collagen hydrogel, has been shown to promote axonal regeneration in vivo [1]. Decellularised extracellular matrices can create a pro-regenerative environment [2]. We hypothesised that EngNT fabricated from decellularised matrix hydrogels will be beneficial for peripheral nerve repair. Three specific ECM hydrogels were screened in vitro for ability to promote neurite extension, with one material then tested in vivo.

Methods

ECM hydrogels were biochemically and mechanically characterised. Cellular alignment was assessed using confocal microscopy 3D image analysis. Rat dorsal root ganglia were harvested and seeded onto stabilised EngNT sheets. Neurite extension was visualised using fluorescence microscopy. A 10 mm rat sciatic nerve transection model was used to compare ECM EngNT with collagen I derived EngNT alongside an empty tube and nerve autograft.

Results

ECM hydrogels differed in stiffness, gelation, and strain responses, as well as sGAG and dsDNA content. Cellular alignment was observed in EngNT made using bone ECM (B-ECM) and small intestinal submucosa ECM (SIS-ECM), but not in liver ECM (LIV-ECM). In vitro neurite extension was observed on all EngNT sheets. Directional outgrowth was exclusive to B-ECM, SIS-ECM, and collagen I EngNT. B-ECM EngNT and collagen I EngNT had comparable in vivo axonal regeneration.

Discussion and conclusions

Hydrogels varied in mechanical properties and biochemical composition, likely affecting cell behaviour. DRG neurite outgrowth on B-ECM was comparable to that of collagen based EngNT and supported regeneration in an in vivo nerve injury model.

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Abstract **#1639**

FETAL NUCLEUS PULPOSUS – BASED SCAFFOLDS: A NOVEL STRATEGY TO TRIGGER IVD REGENERATION

Morena Fiordalisi[1,2,3], Joana Ferreira[1,2,3], Ana João Silva[1,2], Marta Pinto[4], Cláudia Ribeiro-Machado[1,2], Mário Barbosa[1,2,3], Raquel Gonçalves[1,2,3], Joana Caldeira[1,2]

[1] *I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

[2] *INEB - Instituto de Engenharia Biomédica, Porto, Portugal*

[3] *ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*

[4] *CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*

Corresponding author's email: fiordalisi@i3s.up.pt

Tissue engineering strategies, particularly the use of decellularized extracellular matrix (ECM)-based biomaterials, have emerged with undeniable promise for IVD regeneration. Independently of human or animal origin, donor age can influence the therapeutic success of tissue decellularized scaffolds [1]. We have shown that fetal nucleus pulposus (NP) ECM is enriched in pro-regenerative proteins (Collagen XII and XIV), when compared with adult and aged animals [2]. Thus, we propose to evaluate the ability of fetal NP-based scaffolds to promote IVD regeneration by ECM remodeling.

Fetus, young and old bovine NPs were decellularized using an SDS-based protocol and characterized for DNA presence and biochemical, structural and mechanical properties. Adult bovine NP cells were cultured ex vivo on decellularized matrices from fetus, young and old NPs. After 7 days, cell survival, metabolic activity and ECM production (Collagen type II and Aggrecan) were analyzed at molecular and protein level.

As results, an SDS-based protocol enabled successful cell removal, while preserving ECM architecture and composition. Fetal and young NPs matrices were more effective in promoting NP cell viability and adhesion than old matrices. Finally, NP cells cultured in fetal matrices promoted higher Aggrecan expression both at mRNA and protein level.

In conclusion, all NP decellularized matrices supported adult NP cell culture. However, fetal NP scaffolds were shown to have an increased capacity to promote ECM synthesis. This is the first report demonstrating a higher pro-regenerative potential of fetal NP matrices, thus opening new avenues for the development of IVD therapeutic strategies.

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Abstract **#1640**

BIOMIMETIC THIN FILM SCAFFOLDS SUPPORT RENAL CELL-BASED FILTRATION AND REABSORPTION

Thomas Gallegos$_P$[1], Robert Hesse[1], Charles Klassen[1], Daniel Cheng[1], Gita Kiaee[1], Susan Nelson[1], Brock Reeve[1,2], Harald Ott[1,3,4]

[1] *IVIVA Medical*

[2] *Harvard Stem Cell Institute*

[3] *Massachusetts General Hospital*

[4] *Harvard Medical School*

Corresponding author's email: cklassen@ivivamedical.com

Intro: End stage renal disease affects millions of patients worldwide. Allotransplantation is the only curative treatment, yet is only accessible to a fraction of patients. Hemodialysis does not adequately replace lost renal function. Engineered kidney-replacing devices could be an alternative, however, excepting native extracellular matrix, no biologic scaffolding system to support cell-based filtration and absorption has yet been developed.

Methods: Biomimetic thin film scaffolds were fabricated by 3D printing, embedding, and leaching opposing networks of sacrificial material across 5µm thick microporous biologic membranes. The separated channel systems were co-seeded with primary glomerular microvascular endothelial cells and immortalized podocytes (glomerular grafts, N=3), or human umbilical vein endothelial cells and immortalized proximal tubule epithelial cells (tubular grafts, N=4) to recapitulate glomerular and proximal tubule anatomy and physiology.

Results: Scaffolds supported cell engraftment, polarization, and barrier formation in vascular and epithelial channels, enabling filtration and reabsorption. Acellular scaffold vascular perfusion at 30mmHg resulted in a flow rate of 5.82mL/min/cm2 (N=9), producing filtrate at 8.01µl/min/cm2 of membrane. Confluent glomerular grafts produced filtrate at a rate of 3.76µL/min/cm2 (48h, N=3). At 1 week, mature tubular grafts retained 96.8% of Inulin-FITC in the tubular epithelium (N=3). At maturity, glucose was transported into the vascular channel at a 24 hour rate of 0.11mg/mL/cm 2 (N=4).

Discussion: Biomimetic thin film scaffolds support formation of perfusable 3D tissues and higher level renal cell based functions such as filtration and reabsorption in vitro. At scale, cellular constructs could enable fabrication of a fully biologic implantable renal replacement device.

Abstract **#1641**

FROM THE MECHANOBIOLOGY OF THE GLIAL SCAR TO THE TREATMENT OF MULTIPLE SCLEROSIS

Ana Paula Pego[P][1,2,3,4], Eva D. Carvalho[1,2,4], Miguel Morais[1,2], Helena Ferreira[1,2], Mariana C. Silva[1,2,3], Marco Araújo[1,2], H. H. Hubbe[5], S. Allen[6], Juliana R Dias[7], Eduardo Mendes[5], Nuno Alves[7], Laura J. Suggs[6], Cristina Barrias[1,2]

[1] *I3S - Institute for Research and Innovation in Health, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal*

[2] *INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal*

[3] *ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal*

[4] *FEUP - Faculdade de Engenharia da Universidade do Porto, Portugal*

[5] *Delft University of Technology, Chemical Engineering Department, The Netherlands*

[6] *University of Texas at Austin, Biomedical Engineering Department, USA*

[7] *Centro para o Desenvolvimento Rápido e Sustentável do Produto (CDRSP), Instituto Politécnico de Leiria, Portugal.*

Corresponding author's email: apego@i3s.up.pt

Multiple sclerosis (MS) represents the most frequent demyelinating disease affecting young people. Although there are oligodendrocyte precursor cells (OPCs) capable of differentiating in oligodendrocytes and producing myelin sheaths in denuded axons, the process of remyelination fails with disease progression leading to irreversible functional failure. Growing evidences suggest that matrix rigidity plays a crucial rule throughout OPC differentiation and oligodendrocyte myelination by unbalancing the intra/extracellular forces.

Here we propose the use of a “tool-box” by constructing a 3D tissue engineered model of an CNS scar exploring nanomaterials(1). The platform consists on a modified alginate (ALG) matrix able to recreate the 3D environment of the glial scar. ALG hydrogels were produced by combination of ALG formulations with cell adhesive moieties and metalloproteinase-cleavable sequences (PVGLIG). OPC metabolic activity increased for matrices containing high concentration of PVGLIG and, consequently, low stiffness values. This was accompanied by the increase of MBP+ cells and the complexity of cellular morphology at D5, D9 and D14 of differentiation. Astrocytes were found to rapidly extend long processes and create an astrocytic network with increased cellular complexity for high PVGLIG content matrices, acquiring an astrogliotic-like phenotype in response to a cocktail of cytokines. The crosstalk between these cells and the effects of the astrogliosis processes on oligodendrocyte differentiation is being investigated. The final aim is to setup a high-throughput platform to study possible targets and drug candidates involved in the modulation of oligodendrocyte differentiation that can ultimately be explored in the treatment of MS.

Work funded by the UTAustin-Portugal Program.

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Abstract **#1644**

PERFORMANCE OF CONSTRUCTS DERIVED FROM DECELLULARIZED PERICARDIAL EXTRACELLULAR MATRIX IN URINARY BLADDER RECONSTRUCTION IN NEW ZEALAND RABBITS

Guadalupe Hortensia Luévano-Colmenero[P][1], Birzabith Mendoza-Novelo[1], Juan Vargas-Mancilla[2]

[1] *División de Ciencias e Ingenierías, Universidad de Guanajuato, León, Mexico*

[2] *Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato, Instituto Politécnico Nacional, Silao de la Victoria, México*

Corresponding author's email: gh.luevanocolmenero@ugto.mx

Constructs derived from pericardial ECM mesh material covered with porcine small intestine submucosa ECM gel and allogeneic vesical cells and platelet gel were assessed in the reconstruction of rabbit urinary bladder defects. Bovine and porcine pericardial tissue was decellularized by a method described previously. Porcine small intestine submucosa tissue was decellularized and hydrolyzed to render a pre-gel that was combined with water-soluble oligourethanes and silica. Some mechanical, physicochemical, and biological characteristics of the constructs were tested envisaging applications in bladder tissue engineering. The functionality of neobladder was assessed by histological, cystometric and mechanical tests. The urine capacity after 16 weeks of implantation was of 64-75% and 18-46%, relative to the control, when rabbit bladder was repaired with constructs derived from bovine and porcine pericardial ECM, respectively. After 17-week implantation, the integration and formation of neobladder was observed, as well as a complete regeneration of urothelium with presence of smooth muscle like the native bladder. We also found the formation of bladder stones, which can be related to the high levels of urate in the urine; that dissolving with the pH promotes the CaOx precipitation by “salting-out” and induces CaOx crystal formation, causing the urolitiasis. Constructs composed of bovine pericardial ECM mesh material covered with ECM gel and allogeneic vesical cells deserve further study in search of suitable alternatives for the reconstruction of defects in the urinary bladder.

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Abstract **#1645**

AN IN VITRO 3D STEM CELL DERIVED MICROFLUIDIC MODEL OF CARDIAC VENTRICULAR EJECTION ENABLED BY TWO PHOTON DIRECT LASER WRITING

Christos Michas$_P$[1], Pranjal Nautiyal[2], Arvind Agarwal[2], Alice White[3], Christopher Chen[1,4]

[1] *Boston University, Department of Biomedical Engineering, Boston MA USA*

[2] *Florida International University, Department of Mechanical and Materials Engineering, Miami FL USA*

[3] *Boston University, Department of Mechanical Engineering, Boston MA USA*

[4] *The Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston MA USA*

Corresponding author's email: cmichas@bu.edu

The majority of the existing in vitro cardiac models do not incorporate the temporal load-length patterns observed in the pressure-volume (PV) loop of the human ventricles. Improved models would elucidate the effect of changing hemodynamic loads in the distinct PV phases on cardiac structural and function during development, adaptive remodeling and maladaptive remodeling. Here, we fabricate 3D concave tissues that can simulate the ventricular ejection within a microfluidic system. We demonstrate that two photon direct laser writing (TPDLW) is an enabling 3D-printing technique for 3D microscopic scaffolds that are designed to mechanically support embedded contracting induced pluripotent stem cell (iPSC)-derived tissues. Our tissue constructs display notable scaffold deformation and ejection fractions, and recapitulate the response of cardiac tissue to well-characterized chemical compounds. We additionally use TPDLW to fabricate miniaturized passive microfluidic check valves that are sensitive enough to be actuated by the tissue-generated flow. We demonstrate that the valves can regulate and rectify the flow, replicating the unidirectional pumping function of the human ventricles and producing a multiphasic pressure-volume diagram resembling a preliminary PV loop. Our microfluidic system highlights a new path towards advanced in vitro cardiac models that represent more accurately the in vivo heart.

Abstract **#1646**

EMULATING EFFECTS OF RADIATION ON HEMATOPOIESIS IN AN ENGINEERED MODEL OF HUMAN BONE MARROW

Daniel Naveed Naveed Tavakol[P][1], Youngbin Kim[1], Martin Liberman[1], Andrew Harken[2], Guy Garty[2], David Brenner[2], Gordana Vunjak-Novakovic[1]

[1] *Columbia University Department of Biomedical Engineering*

[2] *Columbia University Center for Radiological Research*

Corresponding author's email: dnt2114@columbia.edu

Cosmic radiation is the most serious risk encountered during long missions to the Moon and Mars, and an important concern for patients undergoing radiation therapy. There is a compelling need to understand the exact effects of cosmic radiation, safety thresholds, and mechanisms of tissue damage, in order to develop measures for radiation protection during extended space travel. As the bone marrow (BM) is highly susceptible to radiation damage, serious injury can cause inability to form blood/immune progenitors and eventual BM failure. We describe here (i) a human, patient-specific model of BM that has capacity for hematopoiesis, (ii) the assessment of the effects of cosmic radiation on the hematopoietic niche, and (iii) applications of radioprotective agents (like G-CSF) for mitigating cellular damage. To this end, we engineered our in vitro model by populating decellularized bone scaffolds with osteoblasts, mesenchymal stem/stromal cells, endothelial cells, and cord-blood-derived hematopoietic stem/progenitor cells. Marrow tissues were subjected to doses representative of therapeutic cancer therapy (photon rays, 0-6 Gy) or deep space radiation (neutron rays, 0-2 Gy). Three weeks post-radiation, flow cytometry, histological staining, and cytokine assays were performed, and the early markers of double-stranded DNA damage and molecular changes in lineage fate were identified. At an approximately 4-fold higher rate than in photon ray exposure, neutron rays resulted in decreased proliferation capacity of CD45+ cells, aberrant matrix formation, and tissue necrosis. Using engineered human systems, we hope to provide a test bed for radioprotective therapeutics to circumvent downstream damage during space exploration. (Funding: NASA TRISH FIP0014)

Abstract **#1647**

RAPIDLY PROTOTYPED HUMAN LUNG-ON-A-CHIP FOR DISEASE AND TOXICITY MODELLING AND COUNTERMEASURE DEVELOPMENT

Shiny Rajan[P][1]

[1] *Wake Forest Institute for Regenerative Medicine*

Corresponding author's email: shrajan@wakehealth.edu

Recent studies have been involved with developing biomimetic microsystems that can reconstitute major organ functions like the air-liquid interface of lung. These bioinspired in vitro models are more physiologically relevant than 2D models and weakly-transferrable animal models for drug and toxicity studies. Here, we propose a rapid prototyping approach by combination of adhesive-film based microfluidics and 3D printing to scale up chip fabrication and improve reproducibility and consistency. This was accomplished by constructing a microfluidic architecture that features a chamber separated by a porous membrane, each side of which can be accessed by the 3D printer independently. First, we bioprinted the apical bi-layer epithelia consisting of an ECM hydrogel layer with human lung fibroblast (HLF) and then a monolayer of human bronchial epithelial cells on upper surface of the membrane. Then, the endothelial monolayer was bioprinted on the opposing lower surface, thus mimicking the multicellular 3D organization and air-liquid interface of lung. The system is capable for rapid biofabrication and scale up and is compatible with fluorescent/colorimetric plate readers and microscope imaging stages and other equipment designed for tissue culture plates/chambers. High-throughput and non-invasive analysis of parameters like surface liquid pH, transepithelial resistance, metabolic activity, cell viability as well as immunohistochemistry and imaging have been demonstrated with this system. The physiologically relevant lung model is capable of disease and toxicity modeling and development of appropriate drug and medical countermeasures by analyzing post-treatments effects on the organ.

Abstract **#1648**

MINIDYSTROPHINΔH2-R19 PARTLY IMPROVES PTEN/PI3K/AKT SIGNALLING IN DYSTROPHIN-DEFICIENT MYOBLAST

Muhammad Dain Yazid$_P$[1,2], Chen Hung-Chih[3,2]

[1] *Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, UKM Medical Centre, Jalan Yaacob Latiff, 56000 Cheras, Kuala Lumpur, Malaysia.*

[2] *School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom.*

[3] *Academia Sinica, No. 28, Lane 70, Section 2, Yanjiuyuan Rd, Nangang District, Taipei City, Taiwan 115.*

Corresponding author's email: dain@ukm.edu.my

The gene encoding dystrophin is one of the largest genes, and results in a rod-shaped dystrophin protein that is responsible for connecting the cytoskeleton to the extracellular matrix. Therefore, the restoration of this protein is hoped to compensate for these effects. In this study, stable minidystrophin-eGFP tagged transfected myoblasts were used. Both C2C12 (non-dystrophic) and dfd13 (dystrophin-deficient) myoblasts were transfected with ~5.8 kb pCR3.1 eGFP-mini dystrophin (pMDysE) using Lipofectamine® LTX and PLUS™ Reagents. All transfected myoblast were culture in low mitogen condition for 10 days to induce differentiation. The cells were subjected to immunofluorescence analysis and total protein extraction prior to western blotting assay technique. The introduction of minidystrophin into dfd13 myoblasts showed no expression of MyHC and MF-20 throughout the differentiation day Whilst, MyHC and MF-20 were found expressed late in C2C12-minidystrophin when compared to C2C12-eGFP myoblasts. In dfd13-minidystrophin myoblasts, PTEN expression was found to be reduced upon differentiation. Akt activation was found to be significantly increased in undifferentiated dfd13-minidystrophin myoblasts compared to dfd13-eGFP myoblasts. However, its expression was found reduced upon differentiation to levels comparable with dfd13-eGFP myoblasts. Surprisingly, p70S6K showed significant massive activation in dfd13-minidystrophin myoblasts compared to dfd13-eGFP myoblasts. In this study, the introduction of minidystrophin into dfd13 indicates that non-full length dystrophin is insufficient to improved dfd13 differentiation. However, there is some improvement of dfdf13-minidystrophin where Akt is activated at the undifferentiated stage and increases of P70S6 kinase activation during differentiation. MinidystrophinΔH2-R19 partly improves PTEN/PI3kAkt signalling in dystrophin-deficient myoblast.

Keywords

mini-dystrophin; dystrophin-deficient myoblast; muscular dystrophy

Abstract **#1649**

SINGLE-CELL RNA SEQUENCING FOR PULMONARY TISSUE ENGINEERING

Allison Greaney$_P$[1], Micha Sam Brickman Raredon[1], Taylor Adams[1], Jonas Schupp[1], Naftali Kaminski[1], Laura Niklason[1]

[1] *Yale University*

Corresponding author's email: allison.greaney@yale.edu

Single-cell RNA sequencing (scRNAseq) has revolutionized our understanding of native, developing, and diseased organs, and can be further leveraged to characterize engineered tissues. This is particularly useful in lung engineering, as pulmonary tissue is comprised of four cell classes and upwards of two dozen distinct cell types. In this work, we developed scRNAseq analysis pipelines to identify cell type and behavior within our engineered constructs, as well as benchmarking engineered to native lung tissue(1). Our engineered lungs are grown using decellularized rat lung scaffolds that are recellularized in bioreactors with epithelial and mesenchymal cells in the airway compartment, and endothelial cells in the vascular compartment. Lungs are cultured for a week in cell-type specific media under arterial perfusion, with continuous pressure monitoring at the inlets and outlets of the organ to measure changes in flow patterns within the organ in real time. By analyzing our engineered lungs with scRNAseq, we identified distinct and heterogeneous changes in cell phenotype associated with perturbations in culture parameters, such as media composition, perfusion rate, and added cell types (i.e. epithelium, endothelium, and mesenchyme, vs. epithelium alone). We further compared transcriptomic patterns between engineered and native lungs to gauge our progress towards a functional tissue that may perform well on implantation. We have leveraged Seurat, SCANPY, Monocle, and Connectome software packages in our scRNAseq analyses(2). Overall, this work introduces paradigms for applying scRNAseq analysis techniques to engineered tissues, which in turn can provide high-resolution translational roadmaps.

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Abstract **#1650**

BUBBLE-CAPTURING ROTATING WALL VESSEL BIOREACTORS FOR IMPROVED RETINAL ORGANOID FORMATION

Michael A Phelan$_P$[1], Anthony L Gianforcaro[1], Jonathan A Gerstenhaber[1], Anand Swaroop[2], Peter I Lelkes[1]

[1] *Dept. Bioengineering, Temple University, Philadelphia PA, USA*

[2] *Neurobiology, Neurodegeneration & Repair Lab., NEI, NIH, Bethesda MD, USA*

Corresponding author's email: pilelkes@temple.edu

Rotating wall vessel (RWV) bioreactors have been used as microgravity analogs to produce cell spheroids and organoids at a faster rate than in other bioreactor devices and with higher structural and functional fidelity. One of the limitations of traditional RWV systems is their well-documented tendency for air bubble formation during operation. The presence of these bubbles negates key features of the RWV environment, such as zero headspace, low shear, and simulated microgravity We recently described the design, construction, and testing of a novel RWV bioreactor capable of continually removing air bubbles from the system without interfering with the fluid dynamics that produce optimized cell culture conditions (1). We modeled this capacity using computational fluid dynamics (CFD) and validated the model with alginate beads and spheroid cultures of A549 human lung adenocarcinoma cells. The areas of spheroids assembled from A549 cells in the novel bioreactor in the presence of air bubbles were an order of magnitude larger than in conventional bioreactors when bubbles were present. We also designed a perfusion RWV bioreactor with improved performance characteristics and differentiated retinal organoids from induced pluripotent stem cells in and outside of the bioreactors (2). The ensuing organoids were evaluated by Imaging and IHC. Organoids generated in both static conditions and the perfused bioreactor exhibit retinal phenotypes as demonstrated with IHC, However, bioreactor cultured organoids developed faster and grew significantly larger. We anticipate that the novel designs will increase experimental reproducibility and consistency when using RWV bioreactors as microgravity analogs.

References

(1)

Abstract **#1651**

LARYNGOTRACHEAL RECONSTRUCTION WITH CARTILAGE PROGENITOR CELLS SEEDED IN A DECELLULARIZED MATRIX CONSTRUCT

Paul Gehretp[1], Soheila Aliakbarighavimi[2,1], Riccardo Gottardi[1,2,3]

[1] *University of Pennsylvania*

[2] *Children's Hospital of Philadelphia*

[3] *Fondazione Ri.MED*

Corresponding author's email: gehretp@seas.upenn.edu

Laryngotracheal reconstruction (LTR) is often required following trauma, tumor resection, or as treatment for subglottic stenosis. Engineered cartilage has been proposed as an alternative to autologous cartilage. This would avoid donor site morbidity and the frequent risk of insufficient autologous cartilage for a successful LTR. For successful translation, a cartilage engineering approach should enable sufficient initial mechanical strength in the engineered constructs with a relative short in vitro maturation time (<1 months) so as to match the clinical timeframe. Decellularized allogeneic or xenogeneic extracellular matrix (ECM) has been a promising approach to engineer almost any organ. In this work we combine decellularized ECM with ear cartilage progenitor cells (eCPCs) that can be harvested in a minimally invasive manner. Our preliminary data show that ECM can indeed be easily recellularized eCPCs to achieve density (~400 cell/mm2) and distribution that match that of native cartilage within 2 weeks. Furthermore, the dynamic (~600 kPa) and bulk (~100kPa) mechanical properties of the eCPCs-ECM match those of native cartilage. Additionally, as the eCPCs differentiate, the GAG and collagen content increases and is comparable to native cartilage. Furthermore, we have shown that eCPCs produce better cartilage than bone marrow derived mesenchymal stem cells, with a lower incidence of calcification and a gene expression profile superior to that of expanded chondrocytes. We have tested the eCPCs-ECM cartilage constructs in a rabbit model of laryngotracheal reconstruction with successful functional outcomes and animal survival. Our findings provide strong pre-clinical evidence of functional cartilage repair using a eCPCs-ECM.

Abstract **#1652**

DECELLULARIZED DENTIN EXTRACELLULAR MATRIX FOR 3D BIOPRINTING

Marcela Junqueira Bernardes$_P$[1], Giovanna Sarra[2], Kethylin Ferreira Brolio[1], Gabriel Romero Liguori[3], Vitor Sinkunas[3], Itamara Itagiba Neves[4], Luiz Felipe Pinho Moreira[3], Hector Caballero-Flores[2], Stella Moreira[1,3]

[1] Post Graduation Program, School of Dentistry, Ibirapuera University, São Paulo, SP, Brazil

[2] Department Restorative Dentistry, School of Dentistry, University of Sao Paulo (USP), São Paulo, SP, Brazil.

[3] Laboratory of Cardiovascular Surgery and Circulation Pathophysiology (LIM-11), Heart Institute (InCor), Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil.

[4] Dental Unit, Instituto do Coração (InCor), Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil.

Corresponding author's email: stellam@usp.br

Hydrogel of Decellularized extracellular matrixs (dECMs) represent a promising alternative to develop bioinks that closely mimic the native environment of cells. In the present study, a hydrogel as bioink was developed and characterized from the decellularized dentin extracellular matrix (dECM) to be used in dental tissue engineering. The hydrogel as bioink was characterized in terms of biochemical composition and biological properties. Also, determination of decellularization efficiency was investigated upon quantification of DNA content. The accumulation of collagen was quantified to analysis the bioink. Furthermore, we recellularized the bioink with stem cells of apical papilla (SCAPs cells) and its adhesion was analyzed by fluorescence-based assays. The cytotoxicity of bioink was analized by MTT. Our results showed that acellular dECM contained higher detectable collagen contents and the lower content of DNA. We further demonstrate that high retention of collagen gradients were detected and retained in decellularized samples after decalcification and decellularization process. The results pointed that dentin from extracted teeth can be decellularized and highly efficient hydrogel was produced, and the resulting bioink supports the adhesion of SCAPs for 48 hours and were not found to be cytotoxic in 24hrs. The dentin decellularized matrix bioink may be a promising for 3D printing in regenerative dentistry.

Keywords

3D printing; stem cells; tissue engineering

Abstract **#1653**

SIMULATED MICROGRAVITY ENHANCES THE DIFFERENTIATION OF CULTURED MOUSE EMBRYONIC STEM CELLS TOWARDS DEFINITIVE ENDODERM

Peter I Lelkes[P][1], Liat Oss-Ronen[1], Robert A Redden[1]

[1] *Dept. Bioengineering, Temple University, Philadelphia PA, USA*

Corresponding author's email: pilelkes@temple.edu

Directed in vitro differentiation of pluripotent stem cells towards definitive endoderm (DE) is of great translational interest since DE cells can further differentiate into cells of numerous internal organs, such as lung, liver, and pancreas. We hypothesized that culturing mouse embryonic stem cells (mESCs) under simulated microgravity (SMG) conditions in Rotating Wall Vessel Bioreactors (RWV-BRs) will enhance directed DE differentiation. To test our hypothesis, we cultured the cells for 6 days in two dimensional (2D) monolayer colony cultures, or as embryoid bodies (EBs) in either static conditions or, dynamically, in RWV-BRs. We used flow cytometry and qPCR to analyze the expression of marker proteins and genes, respectively, for pluripotency (Oct3/4) and the induction of mesendodermal (Brachyury T), endodermal (FoxA2, Sox17, CxCr4), and mesodermal (Vimentin, Meox1) lineages. Culture as EBs in maintenance media (MM) in the presence of LIF, in static or SMG conditions, induced the expression of some of the differentiation markers, suggesting heterogeneity of the cells. This is in line with previous studies showing that EB culture will initiate mESC differentiation even without supplementing differentiation factors to the media. Culturing mESCs under SMG conditions in RWV-BRs in differentiation medium (DM) upregulated Brachyury T and all of the DE markers, and reduced Oct3/4 expression, indicating the advantage of dynamic cultures in RWV-BRs to specifically enhance directed DE differentiation. Given the reported discrepancies between the SMG conditions on earth and actual microgravity conditions, future experiments in space flight are required to validate the effects of reduced gravity on mESCs differentiation.

Abstract **#1655**

FRESH 3D BIOPRINTING OF SURGICAL TISSUE TRAINING MODELS USING NATIVE BIOMATERIALS

Andrew Lee[P][1], Riley Patten[1], Thomas Hinton[1]

[1] *FluidForm*

Corresponding author's email: andrew@fluidform3d.com

Surgical simulation has emerged in recent years as a critical path to surgical success. The ability to accurately simulate surgical environments has been shown to reduce costs and risks of operations in an ever-evolving landscape of medical devices, surgical techniques, and cross-functional surgical teams. Recent efforts to simulate surgery has largely involved hands-on training modules that are designed for specific tissue types in individual operations. 3D printing has been an important tool for recreating the highly specific surgical tissue environments. Models of organ systems has been successfully 3D printed using a variety of synthetic resins. However, these models are limited by the materials they choose in that they lack comprehensive surgical feedback that involves simultaneous visual and tactile response. Here, we show the ability to print large-scale tissue simulation constructs with macroscale and microscale control of geometry, using native biomaterials tailored for tissue mechanical properties. Using the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting technology, we demonstrate the ability to accurately pattern native biomaterials such as collagen to create patient-specific constructs that offer life-like simulation of tactile and visual response. The ability to create multi-material constructs with patterned regions of mechanical properties is shown to better mimic the surgical environment for a variety of tissue types, ranging from heart valves, adult and pediatric tracheas, intervertebral discs, and ligaments and tendon. The use of these printed constructs from native biomaterials will provide a more accurate, on-demand simulation that should drastically reduce surgical training cost and increase core surgical competencies.

Abstract **#1657**

ANTI-INFLAMMATION EFFECT OF INFRAPATELLAR FAT PAD MESENCHYMAL STEM CELL FOR OSTEOARTHRITIS TREATMENT

Yu-Chun Chen[P][1,2], Chih-Hung Chang[1,3]

[1] *Department of Orthopedics, Far Eastern Memorial Hospital, New Taipei City, Taiwan*

[2] *College of General Studies, Yuan Ze University, Taoyuan, Taiwan*

[3] *Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Taoyuan, Taiwan*

Corresponding author's email: f94548002@gmail.com

Osteoarthritis (OA) is one of the most common joint disorders related to cartilage. Taking NSAIDs, hyaluronic acid (HA) or platelet-rich plasma (PRP) injection are common non-operative treatment for OA. Recently, researchers found pro-inflammatory cytokines, such as interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), plays critical roles in the pathophysiology of OA, and they affect both quantity and quality of the cartilage matrix. Among various cell-based therapies for OA treatment, mesenchymal stem cell (MSC) injection is the most high-profile therapy method. It can exist in various human tissue including bone marrow, adipose tissue, synovium and infrapatellar fat pad (IPFP). In the study, we evaluated the anti-inflammation effect of IPFP-MSC by chondrocyte or peripheral blood mononuclear cell (PBMC) co-cultivation. Results showed that IPFP-MSC have highly proliferative and differentiate capability, they can express CD73, CD90, CD105, lack expression of CD11b, CD19, CD34, CD45, and express aggrecan, type II collagen and SOX-9 mRNA. When we co-cultured IPFP-MSC and PBMC together, we found IPFP-MSC can inhibited the proliferation of PBMC and decrease TNF-α synthesis in IPFP-MSC/macrophage co-culture system. Moreover, we also found that IPFP-MSC could down regulated inflammation related mRNA of chondrocyte including IL-1β, TNF-α, IL-6 and COX2 under the stimulation of 10 ng/mL IL-1β in IPFP-MSC/chondorcyte co-culture system. Through this study, we proved that IPFP-MSC possess well anti-inflammation effect, and it may further improve cartilage repair environment in OA knee. The findings of this work can provide the direction of cell therapy or drug development for OA treatment.

Keywords

infrapatellar fat pad; mesenchymal stem cell; inflammation

Abstract **#1658**

ANALYSES OF PERIOSTEAL CELL-SEEDED B-TRICALCIUM PHOSPHATE CONSTRUCTS BY RAMAN SPECTROSCOPY

Alexander Dorothea$_P$[1], Felix Umrath[1], Sophie-Maria Kliesch[2], Jürgen Geis-Gerstorfer[3], Siegmar Reinert[1]

[1] *University Hospital Tübingen, Dept. of Oral and Maxillofacial Surgery*

[2] *Quality Analysis GmbH, Nürtingen*

[3] *University Hospital Tübingen, Section Medical Materials Science & Technology*

Corresponding author's email: dorothea.alexander@med.uni-tuebingen.de

Our study investigates the feasibility of the Raman technique for the monitoring of mineralization formed by 3D-cultured cells.

Jaw periosteal cells (JPCs) were grown within β-tricalcium phosphate (β-TCP) as well as polylactic acid coated (β-TCP/PDL) constructs for 17 days under undifferentiated and osteogenic conditions. Before cell colonialization compressive strength (N/mm2) of uncoated/coated β-TCP scaffolds was determined. Raman spectra of 3D-cultivated JPCs from 3 donors were recorded. Cell-seeded constructs were histologically analyzed.

PDL-coated β-TCP constructs showed a significantly higher compressive strength in comparison to that of uncoated scaffolds. Histological analyses showed a relatively homogenous cell colonialization of both materials. On the surface of the composite material, a thin but very dense cell layer was detected, whereas the uncoated β-TCP core material seemed to be covered by a thicker layer of loosely distributed cells.

Recorded Raman spectra from cell-free scaffolds showed that the composite material could be clearly identified. On the surface of uncoated scaffolds, the amount of free phosphate groups was shown to be much higher than on the surface of PDL-coated scaffolds. Due to strong scattering of the material, the cell-specific hydroxyapatite peak was covered by material-specific peaks. However, cell material could be clearly identified by two specific peaks.

The obtained data show clear material and cell specific peaks. However, the cell-specific hydroxyapatite peak is difficult to be identified due to strong scattering of material. Further analysis should optimize the process of data analysis in order to be able to detect cell mineralization within ceramic scaffolds using Raman spectroscopy.

Keywords

periosteal cell-seeded 3D constructs; β-tricalcium phosphate; Raman micro-spectroscopic analyses

Abstract **#1659**

PROTECTIVE EFFECT OF EXOSOMES DERIVED FROM MESENCHYMAL STEM CELLS ON MOUSE ISLET SURVIVAL AND FUNCTION

Somayeh Keshtkar$_P$[1,2], Negar Azarpira[2], Mohammad Hossein Ghahremani[1]

[1] *Department of molecular medicine,school of advanced technologies in medicine, Tehran university of medical sciences, Tehran, Iran*

[2] *Transplant research center, Shiraz university of medical sciences, Shiraz, Iran*

Corresponding author's email: somayehkeshtkar@gmail.com

Islet cell death and loss of function after isolation and during the culture period is considered a main obstacle to successful islet transplantation outcomes (1). Mesenchymal stem cells (MSC) have been shown protective effect on islet viability and function through paracrine interactions and/or direct contact both in the culture period and after transplantation (2). The paracrine function of MSCs are mediated by the secretion of soluble factors and release of extracellular vesicles including exosomes(3). Studies on the effect of extracellular vesicles on islets are very limited. In the present study, we examined the effect of exosomes derived from MSCs on the survival and function of isolated mouse islets. Isolated islets were cultured for 72 hours with MSC-derived exosomes (MSC-Exo), MSCs, or MSC- conditioned media without exosomes (MSC-CM-without-Exo). Islet viability evaluated by fluorescent staining. Apoptosis investigated by TUNEL assay and expression of anti-apoptotic BCL-2 and PI3K and pro-apoptotic BAD and BAX genes in the cultured islets. Beta cell function evaluated by insulin gene expression and protein secretion. The MSCs and MSC-Exo, but not the MSC-CM- without-Exo, significantly decreased the percentage of apoptotic cells and increased islet viability following the downregulation of pro-apoptotic genes and upregulation of pro-survival factors, as well as the promotion of insulin gene expression and secretion. Therefore, exosomes derived from MSCs are as efficient as MSCs for attenuating cell death and improving islet survival and function, suggesting a potential strategy for ameliorating islet transplantation outcomes.

Keywords

Exosome; Mesenchymal stem cell; Islet cell

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Abstract **#1660**

A COMBINATORIAL MASS SPECTROMETRY AND MULTIPLE ELISA APPROACH WARRANTS THOROUGH CHARACTERIZATION OF THE HUMAN PANCREATIC MATRISOME

Giuseppe Orlando[P1], Amish Asthana[1,1], Riccardo Tamburrini[1], Steve Walker[1], Deborah Chaimov[1], Emmanuel Opara[1], Shay Soker[1], Mark Van Dyke[2], Domenica I. Marino[3]

[1] *Wake Forest University School of Medicine*

[2] *University of Arizona*

[3] *Ohio State University*

Corresponding author's email: gorlando@wakehealth.edu

Interactions between the pancreatic extracellular matrix (ECM) and islet cells are known to regulate multiple aspects of islet physiology, including survival, proliferation, and glucose-stimulated insulin secretion. Recognizing the essential role of ECM in islet survival and function, various engineering approaches have been developed that aim to utilize ECM-based materials to recreate a native-like microenvironment. However, a major impediment to the success of these approaches has been the lack of a robust and comprehensive characterization of the human pancreatic proteome. Herein, by combining mass spectrometry (MS) and multiplex ELISA, we have provided an improved workflow for the in-depth profiling of the proteome, including minor constituents that are generally underrepresented. Moreover, we have further validated the effectiveness of our detergent-free decellularization protocol in the removal of cellular proteins and retention of the matrisome. It has also been established that the decellularized ECM and its derivatives can provide more tissue-specific cues than traditionally used biological scaffolds and are therefore more physiologically relevant for the development of hydrogels, bioinks and medium additives, in order to create a pancreatic niche. The data generated in this study would contribute significantly to the efforts of comprehensively defining the ECM atlas and also serve as a standard for the human pancreatic proteome to provide further guidance for design and engineering strategies for improved tissue engineering scaffolds.

Abstract **#1661**

MONITORING THE SYNTHESIS AND DEGRADATION PROFILES OF COLLAGEN IN EXTRACELLULAR MATRIX FROM ENGINEERED CARTILAGE USING FLUORESCENCE LABELING WITH AZIDE-PROLINE BOTH IN VITRO AND IN VIVO

Jinho Park[P][1,2], In Su Park[2], Moon Suk Kim[1], Byoung-Hyun Min[1,2,3]

[1] *Department of Molecular Science & Technology, Ajou University, Republic of Korea*

[2] *Cell Therapy Center, Ajou University Medical center, Republic of Korea*

[3] *Department of Orthopedic Surgery, School of Medicine, Ajou University, Republic of Korea*

Corresponding author's email: bmejinhopark@gmail.com

In cartilage tissue engineering, it is important to determine the distribution and degradation profiles of implanted engineered cartilage tissues. Observing collagen dynamics is helpful to understand the metabolism of cartilage tissues. However, studies of in vivo follow-up of collagen have not been conducted enough due to limitations of existing labeling methods. Thus, in this study, we used azide-proline metabolic labeling method because this method is bioorthogonal and suitable for dense and complex tissues as well as cells cultured in vitro.

Firstly, to observe the collagen secretion according to releasing time and density of extracellular matrix in a single cell, the cells were encapsulated in agarose and then cultured in chondrogenic medium. Labeled molecules were widely distributed over time and narrowly distributed as the density of the agarose gel increases. Next, we incorporated azide-proline into the engineered cartilages. Then fluorescence imaging and collagen contents assays were performed. Dependently on time, the amount of collagen and the fluorescence intensity increased correspondingly. To evaluate real-time monitoring the azide-proline-labeled collagen in engineered cartilage in vivo, we applied it to a nude mouse subcutaneous window chamber model. The amount of collagen also corresponded to the fluorescence intensity.

In conclusion, using bioorthogonal and specific labeling methods, collagen synthesis, distribution, and degradation profiles were observed in real-time at both the single cell and tissue level. The applications presented in this study is expected to be useful for understanding collagen metabolism in various other tissues containing collagen as well as engineered cartilage tissues in vitro and in vivo.

Keywords

cartilage; click chemistry; scaffold-free

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Abstract **#1662**

3D PRINTED SCAFFOLD BASED ON GELMA/GELATIN HYDROGEL FOR FULL THICKNESS WOUNDS

zahra pazhouhnia$_P$[1,2], Nasrin Lotfibakhshaiesh[1]

[1] Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Iran

[2] Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Corresponding author's email: zahrapazhohniya@yahoo.com

The conventional skin tissue engineering approaches have developed several skin substitute products such as Integra and Matriderm. Moreover, these commercial skin products must be changed multiple times during healing process. Nowadays, 3D bioprinting, a high-throughput technology for precise fabrication of 3D construction,is used to raise applicability and function of cell-laden scaffolds.Currently,natural hydrogels used to support epidermal regeneration are mainly gelatin-based, which mimic the natural dermal extracellular.GelMA,which forms covalently cross-linked hydrogels under UV light exposure with the presence of a photoinitiator, has recently gained increasing attention, especially in the field of biomedical applications. GelMA is synthesized by the direct reaction of gelatin with MA in phosphate buffer at 50 °C. This reaction introduces methacryloyl substitution groups on the reactive amine and hydroxyl groups of the amino acid residues. We present a method to create multi-layered engineered tissue composites consisting of human skin fibroblasts and keratinocytes which mimic skin layers. In this study multiple layers of fibroblast containing GelMA hydrogel precursor will be print and crosslink using LAP,constituting the dermal layer. Keratinocytes will be sequentially printed and then, by collagen, these two layers of print will be attached to form two layers of skin.GelMA is developed from a natural polymer gelatin via one-step chemical modification. GelMA has a lot of advantage such as high adhesive, high biocompatibility, and high gelation but in return has a limitation such as low mechanical properties, non-elasticity and fast degradation rate in vivo. To eliminate these constraints, various concentrations of gelatin were added to GelMA before printing.

Keywords

GelMa ,; 3Dbioprinter; Hydrogel

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Abstract **#1663**

BIOSTIMULATIVE EFFECT OF LASER ON GROWTH OF MESENCHYMAL STEM/STROMAL CELLS IN VITRO

Natalia Siedlecka[P][1], Kaja Mecinska-Jundzill[2], Ewelina Fierek[1], Zuzanna Fekner[1], Arkadiusz Jundzill[1], Marta Rasmus[1], Tomasz Kloskowski[1], Kamil Szeliski[1], Wiesław Bienkowski[3], Rafał Czajkowski[2], Tomasz Drewa[1], Marta Pokrywczynska[1]

[1] Chair of Urology, Department of Regenerative Medicine, Cell and Tissue Bank, Nicolaus Copernicus University in Torun, Ludwik Rydygier Medical College in Bydgoszcz, Poland

[2] Chair of Dermatology, Sexually Transmitted Diseases and Immunodermatology, Faculty of Medicine, Nicolaus Copernicus University in Toruń, Poland

[3] Bienkowski Medical Center, Bydgoszcz, Poland

Corresponding author's email: siedleckanatalia92@gmail.com

Introduction: Human Adipose Derived-Mesenchymal Stromal Cells (hAD-MSCs) are multipotent stromal cells with a high potential application in tissue engineering and regenerative medicine. Laser irradiation of the place where the cells were implanted can stimulate their proliferation, increase the secretion of growth factors and thus increase the therapeutic effect.

Aim: The aim of this study was to evaluate the influence of two lasers: Er:YAG and diode on the growth of hAD-MSCs in vitro.

Material and methods: AD-MSCs were isolated from human subcutaneous adipose tissue. Immunophenotype of AD-MSCs was confirmed by flow cytometry. Multipotency of AD-MSCs was confirmed by differentiation into adipogenic, osteogenic and chondrogenic lineages.

AD-MSCs were irradiated with Er:YAG laser (wavelength 2940nm, frequency 5, 10Hz, doses: 0.1-1.2J/cm2) for 2s and 4s and diode laser (wavelength 635nm and doses: 1- 8J/cm2) for 5, 10, 20, 30 and 40s. Cell viability was analyzed 24 hours after the exposure using MTT assay.

Results: Growth stimulation of AD-MSCs after 5Hz Er:YAG laser exposure, 0.1J/cm2 dose for 4s and 0.3J/cm2 dose for 4s was shown in comparison with the control group. Significant growth stimulation of AD-MSCs after diode laser irradiation in doses 1-4J/cm2 was demonstrated compared to the control group.

Conclusions: The presented results indicate that both lasers, Er:YAG and diode can be used to stimulate stem/stromal cell growth in vitro. The biostimulative effect of laser therapy on stromal cells may be used in the future in esthetic dermatology in combined laser and cell therapy.

Abstract **#1664**

ELECTROSPUN SCAFFOLDS FOR OVARIAN TISSUE ENGINEERING: KEY POINTS OF SCAFFOLD-FOLLICLES INTERACTIONS

Liliana Liverani[1], Nathalie Bleisinger[2,3], Inge Hoffmann[2,3], Matthias W. Beckmann[2,3], Ralf Dittrich[2,3], Aldo R. Boccaccini[1]

[1] *Institute of Biomaterials, Dept Materials Science and Engineering, University Erlangen-Nuremberg*

[2] *Erlangen Hospital, Friedrich-Alexander University Erlangen-Nuremberg*

[3] *Comprehensive Cancer Center ER-EMN*

Corresponding author's email: liliana.liverani@fau.de

The application of tissue engineering approach for reproductive organs has been introduced recently (1), raising the interest of the scientific community. Even if the impact on patients' quality of life is very promising, in particular for adult and pediatric oncological patients, only few scaffold fabrication technologies have been investigated for this purpose. Electrospinning technique has been selected for its ability to allow the fabrication of nano and microfibers, which resemble the native morphology of medulla and ovarian cortex. Starting from this biomimetic approach, the use of electrospun scaffolds have been investigated for the support of in vitro ovarian follicles growth. Several requirements need to be fulfilled to maintain the vitality and support the follicles development, in fact previous studies reported that the ovarian follicles need to keep their spherical shape, which can be strongly affected by the substrate used for the in vitro culture. In previous works, we demonstrated that the patterned electrospun fibers are able to provide a suitable support for the growth and development of ovarian follicles, being able to investigate also the infiltration of the follicles inside the scaffolds (2,3). In this work, we further investigate the effect of the fiber diameter, pattern and orientation as well as different polymeric blends on ovarian follicles viability and growth.

Keywords

electrospinning; ovary tissue engineering; ovarian follicles in vitro growth

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Abstract **#1665**

THE IMPACT OF IMAGING MASS SPECTROMETRY ON SKIN RESEARCH – LOOKING INTO THE EPILIPIDOME OF AGING SKIN

Martina Marchetti-Deschmann[P][1,2], Samuele Zoratto[1,2], Christopher Kremslehner[3,2,4], Markus Schosserer[5,2,4], Florian Gruber[3,2,4]

[1] *Institute of Chemical Technologies and Analytics, TU Wien, Vienna, Austria*

[2] *Christian Doppler Laboratory for Skin Multimodal Imaging of Aging and Senescence –SKINMAGINE-, Vienna, Austria*

[3] *Department of Dermatology, Medical University of Vienna, Vienna, Austria*

[4] *Christian Doppler Laboratory on Biotechnology of Skin Aging, Vienna, Austria*

[5] *Department of Biotechnology, BOKU, Vienna, Austria*

Corresponding author's email: martina.marchetti-deschmann@tuwien.ac.at

Lipids are highly diverse biomolecules crucial for the formation and function of cellular membranes, for metabolism, and for cellular signaling. In the mammalian skin, lipids additionally serve for the formation of the epidermal barrier and as surface lipids, together regulating permeability, physical properties, acidification and antimicrobial defense. Recent advances in accuracy and specificity of mass spectrometry have allowed studying enzymatic and non-enzymatic additions and modifications of lipids – the epilipidome –, multiplying the known diversity of molecules in this class. Exposure to frequent oxidative, chemical or thermal stress, injury and inflammation makes it an ideal organ to study epilipidomic dynamics.

We investigated lipid changes in skin equivalents, ex vivo human and animal samples in the context of aging. High resolution mass spectrometry using a 7T MALDI FTICR instrument allowed for accurate mass measurement and therefore immediate lipid identification. In the course of our study we evaluated sample preparation techniques in respect to matrix application but also in the context of multimodal imaging approaches combining MALDI MSI, Raman Imaging and immunohistochemistry. Results were complemented by quantitative lipid profiles from LC-MS/MS.

We found that the relative content of LysoPC and other investigated oxidized phospholipids was upregulated in aging skin and these modified lipid species could specifically be localized in dermal tissue by MALDI MSI. Multimodal image analysis has been performed that correlates these findings with enzymatic activity, Raman fingerprints and immunohistological parameters of senescent cells.

Abstract **#1666**

POLY (L-LACTIDE) NANOFIBERS COATED WITH MINERAL TRIOXIDE AGGREGATE ENHANCE ODONTOGENIC DIFFERENTIATION OF DENTAL PULP STEM CELLS

Ehsan Seyedjafari[P][1], Parisa Sanaei-rad[2]

[1] *University of Tehran*

[2] *Qazvin University of Medical Sciences*

Corresponding author's email: seyedjafari@ut.ac.ir

Induction of mineralization in the dental pulp tissue is essential for the success of a pulp capping material for the treatment of pulp exposure. The purpose of the present study was to investigate the attachment, proliferation and odontogenic differentiation of dental pulp stem cells (DPSC) on poly (L-lactide) (PLLA) nanofibers coated with mineral trioxide aggregate (MTA). Electrospinning was used to fabricate polymeric nanofibers and then their surface was coated with MTA. DPSC were isolated from dental pulp and their viability was evaluated on scaffolds and the control group using MTT assay. During odontogenic differentiation, biomineralization, alkaline phpsphatase (ALP) activity, and the expression of odontogenic genes were analyzed. Isolated DPSC showed fibroblast-like morphology with multi-lineage differentiation potential. MTA-coated PLLA (PLLA/MTA) exhibited nanofibrous structure and interconnected pores and also suitable mechanical properties. Similar to MTA, these scaffolds were shown to be biocompatible and support the attachment and proliferation of DPSC. During odontogenic differentiation, biomineralization, ALP activity and odontogenic-related genes were significantly higher on PLLA/MTA scaffolds compared with control groups. Taking together, MTA/PLLA electrospun nanofibers enhanced the odontogenic differentiation of DPSC and showed appropriate characteristics of a pulp capping material.

Abstract **#1667**

PERIPHERAL NERVE REGENERATION USING BIO3D NERVE CONDUITS CREATED WITH A BIO 3D PRINTER

Ryosuke Ikeguchi$_P$[1], Tomoki Aoyama[1], Makoto Ikeya[2], Takashi Noguchi[1], Hirofumi Yurie[1], Hisataka Takeuchi[1], Sadaki Mitsuzawa[1], Chengzhu Zhao[2], Maki Ando[1], Koichi Yoshimoto[1], Yudai Miyazaki[3], Shizuka Akieda[3], Koichi Nakayama[4], Shuichi Matsuda[1]

[1] *Department of Orthopaedic Surgery, Kyoto University*

[2] *Department of Clinical Application, Center for iPS Cell Research and Application, Kyoto University*

[3] *Cyfuse Biomedical K.K.*

[4] *Center for Regenerative Medicine Research, Saga University*

Corresponding author's email: ikeguchir@me.com

The repair of peripheral nerve injuries with nerve gaps requires autologous nerve grafts to bridge the injured nerve stumps. Autologous nerve grafting is the gold-standard method with good nerve regeneration potential due to the Schwann cells that the graft supplies along with its scaffold function. However, sacrificing healthy nerves leads to donor site morbidities such as loss of sensation and pain. To overcome these disadvantages, artificial nerve conduits have been developed. The three main components required for regeneration are a scaffold, cells and growth factors. Because artificial nerve conduits provide a scaffold only, their use results in nerve regeneration that is not as good as that permitted by autologous nerve grafts. Researchers have attempted to apply various cells to artificial nerve conduits to improve the results, but previous application methods have had low administration efficiency and the cells have had low survival rates. As an alternative application method, we used a Bio 3D printer (Cyfuse Biomedical K.K.) to effectively apply the kinds of cells that are essential to regenerative medicine in peripheral nerve regeneration. In this presentation, we introduce our method by which peripheral nerve injuries can be repaired using nerve conduits created from various cells using a Bio 3D printer.

Abstract **#1669**

POLYMERIC SCAFFOLDS FOR DIRECT CARDIAC GENETIC REPROGRAMMING

Priyadharshni Muniyandi[1], Vivekanadan Palaninathan[1], Toru Maekawa[1], Tatsuro Hanajiri[1]

[1] *Toyo University*

Corresponding author's email: priyadharshni8612@gmail.com

Regeneration of injured myocardium is critical and challenging. Around the world, more than one million patients suffer myocardial infraction annually, most of whom are affected lack shortage of tissue and organs. Thus, there is a great need for the development of new and innovative method mirroring the naive ECM features to improve the quality of patient's life. To overcome these challenges, the development of in-situ direct reprogramming using genetic and epigenetic factors to develop a functional patch to mimic defined microenvironment for reprogramming hold promise for future. The role of delivery of "in-situ" scaffold mediated miRNA/drug delivery for cardiac regeneration is crucial as it can help to mobilize on the site of injury and trigger the reprogramming capacity.The electrospun PLLA scaffolds, combined with both biochemical and physical cues when implanted to the injured site can unlock the regenerative ability. In turn, this induces and drives the differentiation of targeted cardiac fibroblast cells into functional cardiomyocyte like cells. Based on our previous finding to overcome the limitation of current therapies, PLLA scaffolds were fabricated with engineered factors to negate the off-target effect and safety concern of existing delivery systems as a clinical translation of regenerative medicine demands an effective localized delivery system for direct drug/miRNA delivery. Thus, multi-modal drug delivery systems using polymer-based scaffolds can help to negate this issue by not only co-existing with the tissue by providing intramyocardial cellular environment but also can give precision control of the release of miRNA that may be crucial for direct cardiac reprogramming.

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Abstract **#1670**

TISSUE-SPECIFIC BIOINKS FOR SIMULTANEOUS MICROFLUIDIC-ASSISTED BIOPRINTING OF PANCREATIC ISLETS AND BLOOD VESSELS

Joanna Idaszek[1], Marina Volpi[1], Alessia Paradiso[1], Marta Klak[2], Michał Wszoła[2], Mateusz Wierzbicki[3], Sławomir Jaworski[3], Ewa Sawosz Chwalibóg[3], Wojciech Święszkowski[1]

[1] *Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland*

[2] *Foundation of Research and Science Development, Warsaw, Poland*

[3] *Department of Nanobiotechnology and Experimental Ecology, Institute of Biology, Warsaw University of Life Sciences, Warsaw, Poland*

Corresponding author's email: joanna.idaszek@pw.edu.pl

Transplantation of pancreatic islets is one of the treatment strategies of type 1 diabetes. Despite many advantages, the therapeutic outcome of this procedure is limited due to high islets loss associated with lack of dense vascular network, among others [1]. In this context, 3D multi-material bioprinting (BP) offers the possibility to fabricate heterogeneous structures composed of pancreatic and vascular compartments.

The aim of this work was to develop tissue-specific bioinks for simultaneous microfluidic-assisted BP of pancreatic islets and blood vessels.

To this end, alginate was blended with decellularized pancreatic extracellular matrix, ECM, or fibrinogen to prepare pancreatic and vascular bioinks, respectively. The BP was performed using commercial bioprinter (Bioplotter) equipped in multi-inlet and single coaxial nozzle outlet microfluidic platform. The 3D-BP scaffolds were characterized with respect to shape fidelity, swelling, mechanical properties and diffusion of glucose and insulin. Porcine pancreatic islets and mixture of human mesenchymal stem cells (MSC) and human umbilical vein endothelial cells (HUVEC) were successfully bioprinted. Islet functionality was assessed using GSIS assay and angiogenic potential by staining against CD31 endothelial marker and implantation onto chick chorioallantoic membrane (CAM).

The bioprinting did not affect islets viability but reduced MSC and HUVEC viability to approximately 80%. The islets sustained their functionality as demonstrated by secretion of insulin under glucose stimulation. During culture, HUVEC spread on the surface of the fibers and formed dense layer, as well as vessel-like structures, of CD31-positive cells. Moreover, denser network of capillaries was formed at the CAM interface, suggesting increased angiogenesis.

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Abstract **#1671**

FORMATION OF VARIOUS CALCIUM PHOSPHATE COATINGS WITHIN 3D-PRINTED SCAFFOLDS USING ALKALINE PHOSPHATASE ENZYME

Joanna Idaszekₚ[1], Jakub Jaroszewicz[1], Emilia Choińska[1], Żaneta Górecka[1], Karol Szlązak[1], Anna Hyc[2], Anna Osiecka-Iwan[2], Wojciech Święszkowski[1], Stanisław Moskalewski[2]

[1] *Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland*

[2] *Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland*

Corresponding author's email: joanna.idaszek@pw.edu.pl

Additive manufacturing (AM) has become one of the techniques most intensively utilized for fabrication of 3D porous scaffolds for tissue engineering (TE) applications. In the case of bone TE, the scaffolds are usually made of thermoplastic polymers and polymer-based composites. However, these biomaterials induce fibrous encapsulation at ectopic sites and result in limited bone regeneration. On the other hand, calcium phosphate (CaP) biomaterials are commonly used in orthopaedics both as implants or coatings thereof. One of the advantages of CaPs is that they allow to omit fibrous encapsulation at osseous site due to their ability to directly bond with bone. Here, we present a straightforward method allowing for homogeneous coating of 3D porous scaffolds.

Poly(ε-caprolactone)-based (PCL) samples were fabricated by means of AM using BioScaffolder (SYSENG, Germany) and coated by incubation in medium containing sodium glycerophosphate as alkaline phosphatase (ALP) substrate and bovine ALP. The formulation was further modified by introduction of NaHCO3. The coatings were characterized using SEM, XRD, FTIR and micro-computed tomography and evaluated with respect to adhesion, proliferation and osteogenic differentiation of human mesenchymal stem cells (hMSCs).

The obtained results indicate that the proposed method yielded homogeneous coatings of external and internal regions of 3D porous scaffolds with chemical composition typical for hydroxyapatite. Presence of the coatings significantly enhanced cell adhesion and accelerated osteogenic differentiation, when compared to unmodified samples. The results showed also the positive effect of introduction of bicarbonate on cell adhesion, ALP activity and migration of hMSCs out of the multicellular spheroids.

Abstract **#1672**

BIOHYBRID HYDROGEL WITH ENHANCED ELECTROCONDUCTIVITY ADVANCES MATURATION OF HIPSC-DERIVED CARDIOMYOCYTES

Kaveh Roshanbinfar[P][1], Felix Engel[1]

[1] *Friedrich-Alexander Universität Erlangen-Nürnberg*

Corresponding author's email: k.roshanbinfar@gmail.com

Cardiac tissues derived from human induced pluripotent stem cells (hiPSC) can be utilized for patient-specific therapies and drug screening.[1] However, the immature state of these cells limits their application. To date, external electrical stimulation resulted in the most eminent maturation.[2,3] Here, we developed an electroconductive 3D biohybrid hydrogel involving collagen I, sodium alginate, and PEDOT:PSS. It enhances beating properties and maturation of hiPSC-derived cardiomyocytes to near-adult characteristics without applying external stimulations.

hiPSCs differentiated in cardiomyocytes in 12 days using CHIR-9902 and IWR-1-endo and mixed with the pre-gel solution of collagen I, sodium alginate, and PEDOT:PSS. Constructs were fixed after 11 days and immunostained for sarcomeric α-actinin, cardiac troponin I, connexin 43, and DNA. Beating movies were recorded and analyzed by the Musclemotion plugin by NIH ImageJ software.

Compared to constructs without PEDOT:PSS, the electroconductive biohybrid hydrogels improved the maturation and beating properties of hiPSC-derived cardiomyocytes. These cells formed more condensed structures, exhibited 1.9 µm near-adult[4] sarcomeric length, enhanced beating frequency, higher contraction speed, and greater contraction amplitude.

Our data indicate that these electroconductive biohybrid hydrogels enhance the maturation of hiPSC-derived cardiomyocytes. In conclusion, we provide a new and versatile approach to fabricate engineered cardiac tissues that beat and maturate on their own without external mechanical or electrical stimulations. These constructs can find their potential application in drug testing to reduce the burden on laboratory animals or cardiac disease treatment tissues.

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Abstract **#1673**

3D PRINTED SINGLE-USE BIOREACTORS FOR THE CULTIVATION AND MATURATION OF LARGE BIOPRINTED TISSUES

PETIOT Emma[P][1], Chastagnier Laura[1], Lemarié Lucas[1], Olmos Eric[2], Loubiere Céline[2], Marquette Christophe[1]

[1] *3d.FAB - ICBMS - University Claude Bernard Lyon*

[2] *ENSAIA - Université de Lorraine*

Corresponding author's email: laura.chastagnier@univ-lyon1.fr

In the last 5 years, bioprinting became a highly active scientific area for regenerative medicine applications. Nevertheless, in-vitro production and maturation of large living tissues (in the range of dm3) is still strongly limited by nutrient supply and cell environment control. Each tissue has his own specific geometry / culture rules to maturate. To sustain cell viability and maturation, a tissue-specific design of their culturing processes have to be developed to reach customized large tissues' production. Such bioprocesses and scale-up will have to go through specific and controlled bioreactors modelling to better rationalize parameter impacting the tissues maturation. Our approach was to design and print single-use bioreactors with optimized internal geometry allowing for tissue nutrient perfusion. Such bioreactors were regulated by an external feeding vessel regulated in pH, temperature and oxygen. Bioreactor's design was optimized with Computational Fluid Dynamics (CFD) allowing to describe and chose the medium perfusion flow path within the bioprinted tissue. Several tissues geometry were targeted, either flat dale for dermis models, porous cubes for conjunctive neo-vascularized tissues or tubes for vascular tissues. A comparative study between static and dynamic culture in bioreactor was performed with bioprinted conjunctive tissues. We observed nutrient flow impact on cell growth and maturation through production of extracellular matrix. Perfusion flow rate of 600mL/h was chosen for its compromise between flow heterogeneity (presence of stagnant zone) and a well-established laminar regime. Clear differences between static and dynamic cultures were observed with an enhanced extracellular matrix production while conserving bioprinted tissue internal geometry.

Keywords

Bioreactor 3d printing; tissue bioprinting; computational flow dynamic CFD

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Abstract **#1675**

REGENERATIVE MEDICINE & TISSUE ENGINEERING: CLINICAL TRANSLATION

Frank Luyten[P][1]

[1] *KU Leuven & RegmedXB*

Corresponding author's email: frank.luyten@kuleuven.be

Cell based therapies, classified in Europe as Advanced Therapy Medicinal Products, have been explored for more than 2 decades now as potential therapeutics in a large variety of clinical indications, including the repair of cartilage and bone. Their clinical impact has so far been limited and this is due to many factors such as our lack of understanding of the mechanisms of action, the poor correlation between in vitro assays and in vivo outcome, the lack of robust manufacturing processes and underpowered, poorly designed clinical studies. Moreover, it is widely accepted that the process design and scaling of engineered cell based products at reasonable cost has been challenging. Recent scientific insights in regenerative medicine strategies indicate that the mechanisms by which cell based implants exert their potency in vivo are multiple, not only contributing to the cellular compartment of the repair tissue but also affecting tissue repair by paracrine mechanisms thereby boosting the endogenous repair potential. However in large tissue defects and in compromised conditions, the data indicate that the extrinsic cellular component is required for successful clinical outcomes. Clinical translation involves many challenges including the appropriate animal models for well-defined patients. Examples will be discussed including combination products using adult periosteal cells and Bone Morphogenetic Proteins (BMP) on optimized scaffolds. Furthermore, we have been focusing on the manufacturing of living implants for cartilage and bone repair and generated cartilage tissue intermediates, following a developmental engineering paradigm, to repair large long bone defects and deep osteochondral joint defects in vivo.

Abstract **#1676**

PERSONALIZED STOMACH-ON-A-CHIP FOR DRUG SCREENING

Tae-Eun Park$_P$[1], Hye-Jin Jeong[1], Seong-Ho Kong[2]

[1] *Ulsan National Institute of Science and Technology*

[2] *Seoul National University Hospital*

Corresponding author's email: tepark@unist.ac.kr

Stomach drug development is largely relies on animal models, which often do not provide results that are translatable to human patients. Organ-on-a-chip represents a new avenue in a range of area in preclinical precision drug validation and new drug discovery using microfluidic technology. Additionally, the use of stomach organoid generated from patient's adult stem cells as a cell source for organ-on-a-chip can emulate individual organ-level function. It enables better understanding of the individual pharmacokinetics and pharmacodynamics properties of various medications providing personalized drug discovery opportunities. Here, we report a personalized stomach-on-a-chip lined by stomach epithelium prepared from organoid interfaced with primary gastric fibroblast that recapitulates the secretion of mucus, gastric enzyme, and stomach barrier function. The enhanced function of stomach-on-a-chip was acquired by peristaltic flow, co-culture, and small molecules enhancing the interaction between the cells. Our stomach-on-a-chip showed potential for Helicobacter pylori research showing the advantages for infection studies over gastric organoids and human gastric cell line.

Abstract **#1677**

DEVELOPMENT OF DECELLULARIZED BONE EXTRACELLULAR MATRIX BIOINK FOR ADDITIVE MANUFACTURING OF 3D BONE TISSUE SUBSTITUTES

Joanna Idaszek[P][1], Marina Volpi[1], Wojciech Święszkowski[1]

[1] *Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland*

Corresponding author's email: joanna.idaszek@pw.edu.pl

Conventional tissue engineering (TE) of bone involves fabrication of 3D porous scaffolds, followed by their inoculation with cells. Recently, 3D bioprinting (BP) emerged as a powerful technology for biofabrication of complex heterogeneous tissue substitutes. In contrast to conventional additive manufacturing, 3D-BP operates in cell-friendly conditions and employs deposition of cell-laden aqueous solutions of biopolymers, so called bioinks. Bioinks used currently for TE applications, although resemble the tissues with respect to high water content, lack of tissue-specific biopolymers and growth factors, which leads to suboptimal tissue formation in vitro. Therefore, decellularized exstracellular matrix (dECM)-based bioinks were proposed for bioprinting of tissues and organs.

The goal of this study was to develop a bioink containing solubilized dECM isolated from porcine bone (dbECM). Following demineralization by hydrochloric acid and decellularization with Triton X-100, the obtained dbECM was dissolved by pepsin digestion. The biochemical analysis revealed that the bioink retained the major component of bony ECM, namely collagen, and was cleared of cellular components (DNA content < 30 ng/ mg of dbECM). The rheological measurements demonstrated shear-thinning behavior. Gelation kinetics increased significantly at higher concentrations of dbECM. The printability of dbECM hydrogels was investigated using 3D printer (Bioplotter, Envisiontec) and the printing parameters were optimized to ensure possibly high shape fidelity. DbECM bioink was also tested for cytocompatibility using human mesenchymal stem cells (hMSC). The ability of hydrogels to support hMSC proliferation and to induce osteogenic differentiation was confirmed by means of MTS assay, measurements of ALP activity, histological stainings and microcomputed tomography.

Abstract **#1678**

A MCT - BASED IDENTIFICATION OF DRUG TRANSPORT AND MECHANICAL PROPERTIES OF THE ENGINEERED BRUCH'S MEMBRANE

Marcin Heljak[P][1], Ewa Kijeńska-Gawrońska[2], Adrian Chlanda[3], Jakub Jaroszewicz[1], Carmelo de Maria[4], Giovanni Vozzi[4], Wojciech Swieszkowski[1]

[1] Faculty of Materials Science and Engineering, Warsaw University of Technology, ul. Wołoska 141, 02-507, Warsaw, Poland

[2] Centre for Advanced Materials and Technologies CEZAMAT, Warsaw University of Technology, 19 Poleczki Str., 02-822 Warsaw, Poland

[3] Institute of Electronic Materials Technology, Department of Chemical Synthesis and Flake Graphene, Wólczyńska 133, Warsaw, 01-919, Poland

[4] Research Centre'E. Piaggio'and Dept. of Ingegneria dell'Informazione of University of Pisa, Pisa, Italy

Corresponding author's email: marcin.heljak@pw.edu.pl

Comprehensive examination of electrospun networks (ESNs) made of aliphatic polyesters in terms of mass transport, mechanical behavior and morphology was performed to explore the possibility for using them as the engineered Bruch's membrane (a multilayered cell-free barrier that separates the retinal pigment epithelium (RPE) from the choriocapillaris) being the component of the developed in vitro model of posterior ocular barrier. The analysis was carried out by means of novel micro-mechanical computational models of ESNs using of morphological data extracted from X-ray microtomography (μCT).

Data obtained from μCT scanning was used to create volumetric finite element meshes representing the investigated ESNs. It was proven that the rendered ESNs micro-structural FEM models well resembled the actual structures of the investigated ESNs.

The model of diffusion-convection transport of different species through the investigated ESNs as well as the mechanical model of ESNs stretching were developed. The developed transport model uses convective fluxes calculated in CFD simulation and it was used to simulate transport of selected drugs and oxygen through the investigated ESNs. The obtained computational results were verified against the experimental data.

It was confirmed that the developed modeling tools enabled for verification of fabricated ESNs for using them as the engineered Bruch's membrane. The presented computational approach enables for time as well as the experimental effort saving.

The developed model enables for accurate identification of properties of ESNs made of aliphatic polyesters, however the authors expect that it should be able to identify properties of ESNs fabricated from the other materials.

Abstract **#1679**

FURTHER EVIDENCE THAT PLATELET-RICH PLASMA PROMOTES PERIPHERAL NERVE REGENERATION THROUGH ENHANCING SCHWANN CELLS FUNCTION

yoshihiro sowaP[1], Tsunao Kishida[2], Toshiaki Numajiri[1], Osam Mazda[2]

[1] *Department of Plastic and Reconstructive Surgery, Kyoto Prefectural University of Medicine*

[2] *Department of Immunology, Kyoto Prefectural University of Medicine*

Corresponding author's email: sowawan@koto.kpu-m.ac.jp

Background: Platelet-rich plasma (PRP) contains high concentrations of growth factors that stimulate proliferation and migration of various cell types. Earlier experiments demonstrated that local PRP administration activates Schwann cells (SCs) to improve axonal regeneration at a transected peripheral nerve lesion[1,2]. However, the optimal concentration of human PRP for activation of human SCs (hSCs) has not been determined, while mechanisms by which PRP activates SCs remain to be clarified.

Methods: hSCs were cultured with various concentrations of PRP in 5% FBS/DMEM. Cell viability, microchemotaxis, flowcytometry, and quantitative RT-PCR assays were performed to assess proliferation, migration, cell cycle, and neurotrophic factor expression of the hSCs, respectively. hSCs were co-cultured with neuronal cells to assess their capacity to induce neurite extension. Neutralizing antibodies for platelet-derived growth factor-BB (PDGF-BB) and insulin-like growth factor-1 (IGF-1) were added to the culture to estimate contribution of these cytokines to the hSC stimulation by PRP.

Results: An addition of PRP at 5% strongly elevated proliferation, migration, and neurotrophic factor production of hSCs. Both PDGF-BB and IGF-1 may be involved in mitogenic effect of PRP on hSCs, while PDGF-BB may also play an important role in the migration-inducing effect of PRP. Neutralization of both PDGF-BB and IGF-1 cancelled promoting effect of PRP on neurite-inducing activity of hSCs.

Conclusions: The present findings may suggest the optimal concentration of PRP for hSC stimulation as well as potential mechanisms underlying the activation of hSCs by PRP, which may be quite useful for the PRP therapy for peripheral nerve regeneration.

Keywords

Platelet-rich plasma; Schwann cells; PDGF-BB

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Abstract **#1680**

NEW METHOD OF SCAFFOLD COATING BY SIMPLE, ALKALINE PHOSPHATASE BASED TREATMENT

Jakub Jaroszewicz$_P$[1], Joanna Idaszek[2], Emilia Choinska[2], Karol Szlazak[2], Anna Hyc[3], Anna Osiecka-Iwan[3], Wojciech Swieszkowski[2], Stanislaw Moskalewski[3]

[1] *Warsaw University of Technology*

[2] *Warsaw University of Technology*

[3] *Medical University of Warsaw*

Corresponding author's email: jakubjaroszewicz@wp.pl

We present a novel, based on alkaline phosphatase activity, approach to the production of calcium phosphate coatings on polycaprolactone scaffolds. Enzymatic hydrolysis of sodium glycerophosphate in the presence of calcium chloride led to the rapid formation of coating. Both external surfaces and in porous 3D scaffolds also its internal walls were covered. Since PCL scaffolds are hydrophobic for effective attachment of calcium coating, their surface required modification either by heating or by mild lipase digestion. Effective deposition of calcium coating occurred during first hour of incubation, longer exposition was, however, needed for appearance of crystalline forms, including hydroxyapatite. The new coatings allowed for protein adsorption to the scaffolds and supported cell adhesion, proliferation and osteoblastic differentiation. It appears that phosphatase based strategy may be also applied to scaffold produced from other materials, providing adequate surface activation. Phosphatase method may represent an interesting alternative to the simulated body fluid (SBF) approach for coating formation.

Keywords

Bioactive coating; 3D scaffold; Hydroxyapatite

Abstract **#1681**

PATTERNED HUMAN EPIDERMAL VITRO MODEL USING BIOPRINTING TO MIMIC HETEROGENEITY OF HUMAN SKIN

Maite Rielland$_P$[1], Sabrina Madiedo-Podvrsan[1], Jean-Philippe Belaidi[1], Stephanie Desbouis[1], Youcef Ben Khalifa[1], Lucie Simonetti[1], Jeremie Soeur[1]

[1] *L'Oréal Research and Innovation*

Corresponding author's email: maite.rielland@rd.loreal.com

Skin is a very complex and heterogeneous tissue and mimicking its complexity remains a difficult challenge for Tissue Engineering 1,2. Skin tissue models have been used for decades for the treatment of burns but also for knowledge and cosmetic and pharmaceutic evaluation studies. In that sense, there is an inherent need for more physiological and predictive skin models 3,4. This study aims to use bioprinting technology to deposit in a controlled manner two populations of human keratinocytes to introduce heterogeneity in in vitro epidermal models 1,5. As a first proof of concept, we have validated the concept through a patterned epidermal model in which a same keratinocyte population was divided into two subpopulations, one of which was transduced with GFP. As a second step we proved that pattern models can have a real physiological relevance by generating a heterogeneous model consisting of a filaggrin down-regulated part, considered as one of the epidermal models for Atopic Dermatitis and Ichthyosis Vulgaris pathologies, and its associated control part. This first report of human patterned epidermal models, following predefined designs using bioprinting-based strategy, provides concrete proof of the power of bioprinting to reproduce human skin microanatomy.

Keywords

3D human model; patterning

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Abstract **#1682**

BIOMIMETIC DESIGN STRATEGY FOR MANDIBULAR BONE RECONSTRUCTION USING 3D BIOPRINTING TECHNOLOGIES

Sang Jin Jin Lee$_P$[1], Hyeongjin Lee[1], Carlos Kengla[1], Ickhee Kim[1], James Yoo[1], Anthony Atala[1]

[1] *Wake Forest School of Medicine*

Corresponding author's email: sjlee@wakehealth.edu

Medical imaging and 3D bioprinting strategy converged to allow the fabrication of a complex structure with inner architecture based on patient anatomy and biomimicry. In this study, we tested a biomimetic design based on the architectural arrangement of bone, which provides a porous core for bone ingrowth and a dense external layer resisting fibrous tissue ingrowth. Biomimetic bone constructs were made of a composition of poly(ε-caprolactone) and β-tricalcium phosphate (PCL/TCP) and fabricated on the Integrated Tissue-Organ Printing (ITOP) system. The osteoconductivity of this PCL/TCP bone constructs was confirmed by in vitro osteogenic differentiation using human placental stem cells (hPSCs). We also examined this biomimetic bone construct in a critically sized mandibular bony defect of rabbits. The results showed the increased bone density and volume and new bone formation and maturation with time. Our finding suggests that bone regeneration could be enhanced by the biomimetic bone construct designed to minimize competing for fibrotic tissue forming in the bony defect. We demonstrated the concept that patient-specific anatomy could be translated to 3D bioprinting strategy through medical imaging and image processing software with strong clinical relevance.

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Abstract **#1683**

A NOVEL PHOTO-CROSSLINKABLE KIDNEY ECM-DERIVED BIOINK FOR RENAL TISSUE REGENERATION

Sang Jin Jin Lee$_P$[1], Gabriel Carreno Galeano[1], Mohamed Ali[1], James Yoo[1], Anthony Atala[1]

[1] *Wake Forest School of Medicine*

Corresponding author's email: sjlee@wakehealth.edu

One of the challenges in 3D bioprinting is finding an appropriate bioink that provides a tissue-specific microenvironment supporting the cellular growth and maturation. Accordingly, decellularized extracellular matrix (dECM)-derived hydrogels have been proposed as bioinks for the cell-based bioprinting due to their capability to inherit the intrinsic cues from native ECM. In this study, we developed a photo-crosslinkable kidney ECM-derived bioink (KdECMMA) that could provide a kidney-specific microenvironment for renal tissue bioprinting. Porcine whole kidneys were decellularized through a perfusion method, dissolved in an acid solution, and chemically modified by methacrylation. This KdECMMA-based bioink was formulated and evaluated for rheological properties and printability for the printing process. Afterward, the bioprinted cell-laden constructs were implanted in the kidneys of nude rats for subsequent analysis. The results showed that the bioprinted human kidney cells in the KdECMMA bioink were highly viable and matured with time. Moreover, the bioprinted renal constructs exhibited the structural and functional characteristics of the native renal tissue. We demonstrated the potential of the tissue-specific ECM-derived bioink for cell-based bioprinting that could enhance cellular maturation and eventually tissue formation.

Acknowledgments: This study was supported by NIH/NIBIB (1P41EB023833).

Abstract **#1684**

AN EXTRUSION-BASED BIOINK ARTIFACT FOR QUANTIFYING PRINTABILITY AND AN EXPLORATORY ANALYSIS ON ITS RELATIONSHIPS WITH RHEOLOGICAL PROPERTIES

Gregory Gillispie[P1], Meryem Uzun-Per[1], James Yoo[1], Anthony Atala[1], Muhammad Khalid Khan Niazi[1], Sang Jin Lee[1]
[1] *Wake Forest School of Medicine*
Corresponding author's email: sjlee@wakehealth.edu

The limited availability of bioinks is a key limitation to bioprinting technology. Meanwhile, the bioink development process itself is limited by the methods for assessing printability and a poor understanding of how rheological factors influence printing outcomes. An extrusion bioink-specific artifact has been developed to increase the quantification, comprehensiveness, and standardization of printability assessment. This exploratory study aims to validate the artifact and utilize it to relate various bioink candidates and their rheological properties to their printability. The artifact was designed according to criteria previously established by other 3D printing modalities. Various hydrogels as bioinks were tested on the artifact and rheologically. Measures from the artifact and image analysis aligned well with expected ranges and relative values. Rheologically, the high-performing bioinks demonstrated high storage modulus, low tan(δ), high shear-thinning capabilities, high yield stress, and recovery abilities which were both fast and near-complete. However, no rheological measure alone was correlated with the differences in printability between the various bioinks. In addition to presenting a novel methodology, these results demonstrate the need to take a holistic view of a bioink's rheological properties and the importance of measuring printing outcomes directly.

Acknowledgments: This study was supported by NIH/NIBIB (1P41EB023833).

Abstract **#1685**

SELF-ALIGNED MYOFIBERS IN 3D BIOPRINTED EXTRACELLULAR MATRIX-BASED CONSTRUCT ACCELERATE SKELETAL MUSCLE FUNCTION RESTORATION

Hyeongjin Lee[P][1], WonJin Kim[2], JiUn Lee[2], Kyung Soon Park[1], James Yoo[1], Anthony Atala[1], Geun Hyung Kim[2], Sang Jin Lee[1]

[1] *Wake Forest School of Medicine*

[2] *Sungkyunkwan University*

Corresponding author's email: sjlee@wakehealth.edu

To achieve rapid skeletal muscle function restoration, many attempts have been made to bioengineer functional muscle constructs by applying physical, biochemical, or biological cues. Here, we develop a self-aligned skeletal muscle construct by printing a photo-crosslinkable skeletal muscle ECM (dECM-MA) bioink containing human muscle progenitor cells (hMPCs). To induce the self-alignment of hMPCs in the printed constructs, in situ uniaxially aligned micro-topographical structure is produced by a fibrillation/leaching of poly(vinyl alcohol) (PVA). The in vitro results demonstrated that the synergistic effect of tissue-specific biochemical and topographical cues improve the myogenic differentiation of the printed hMPCs with cellular alignment. This self-aligned muscle construct shows the accelerated integration with neural network and vascular ingrowth in a rodent model of muscle defect injury, resulting in rapid restoration of muscle function. We demonstrate that combined biochemical and topographic cues on the 3D bioprinted skeletal muscle constructs can effectively reconstruct the extensive muscle defect injuries.

Acknowledgments: This study was supported by NIH/NIBIB (1P41EB023833).

Abstract **#1686**

BIOFUNCTIONALIZED ELECTROSPUN VASCULAR SCAFFOLDS FOR REPLACING SMALL-DIAMETER BLOOD VESSELS

Young Min Ju[P][1], Hyeongjin Lee[2], Ickhee Kim[2], Anthony Atala[2], James Yoo[2], Sang Jin Lee[2]

[1] *Wake Forest School of Medicine*

[2] *Wake Forest School of Medicine*

Corresponding author's email: sjlee@wakehealth.edu

clinical need for the development of functional vascular substitutes. In this study, we aimed to develop a biofunctional electrospun vascular scaffold by chemically conjugating antibody and anti-thrombogenic agent. We hypothesized that multiple bioconjugation of endothelial cell (EC)-specific antibodies and anti-thrombogenic agents onto the vascular scaffold could facilitate in situ endothelialization while preventing blood clotting. To achieve this goal, bioconjugation parameters on a vascular scaffold was optimized to provide proper biological properties and structural configuration that enhanced cellular interactions of ECs on the lumen. This biofunctional vascular scaffold was validated in a sheep carotid arterial interposition model. The implanted scaffolds were followed by an ultrasound to evaluate the vessel diameter, as well as patency quantitatively. In vivo experiment showed that these vascular scaffolds maintained a high degree of patency and structural integrity and produced the matured EC coverage. We demonstrated that the generation of the biofunctional vascular scaffold, along with directed immobilization of bioactive molecules, could provide the necessary components for successful vascular tissue engineering applications.

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Abstract **#1687**

A PERFUSABLE VASCULARIZED FULL-THICKNESS SKIN MODEL FOR TOPICAL AND SYSTEMIC APPLICATIONS

Sacha Salameh[P][1], Nicolas Tissot[1], Kevin Cache[1], Joaquim Lima[1], Itaru Suzuki[1], Paulo André Marinho[1], Maité Rielland[1], Jérémie Soeur[1], Shoji Takeuchi[2], Stéphane Germain[3], Lionel Breton[1]

[1] *L'Oréal Research and innovation, Aulnay-sous-Bois, France*

[2] *Department of Mechano-Informatics, Graduate School of Information Science and Technology, The University of Tokyo, Japan*

[3] *Center for Interdisciplinary Research in Biology (CIRB), College de France, CNRS, INSERM, PSL Research University, Paris, France*

Corresponding author's email: sacha.salameh@rd.loreal.com

Skin models are used in the evaluation of efficacy and safety of active compounds and drugs in cosmetic and pharmaceutical research, especially after the ban of the animal testing in cosmetics research in 2013 [1-3]. Many advances have been made in skin engineering but challenges still exist for constructing thick or complex tissues with all in vivo functions [4]. One of the main challenges is vascularization. Given that the diffusion coefficient of oxygen is limited to 200µm in human tissues, skin constructs thicker than 200 µm have a diffusion limitation [5, 6].Therefore, developing perfusable vascular networks is mandatory to improve the functionality and viability of skin grafts as well as the relevance of in vitro applications. To address this issue, our study focused on the development of a perfusable vascularized full thickness skin equivalent with more complex vasculature than existing models. We here combined molding, auto-assembly and microfluidics techniques in order to produce a skin equivalent that recapitulates a mature epidermis, a complex microvascular network and three vascular channels with angiogenic sprouts required for the perfusion of the reconstruct. We then evaluated skin permeability to compounds with different chemical properties and systemic delivery of the Benzo[a]pyrene pollutant. Our results demonstrated that perfusion of the vascular plexus resulted in a more predictive and reliable model in order to assess both topical and systemic applications. This model is therefore aimed at furthering drug discovery and improving clinical translation in dermatology.

Keywords

Reconstructed skin; Vascularization; Perfusion

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Abstract **#1688**

TWO-LAYERED NANOFIBROUS MAT LOADED WITH GROWTH FACTORS, SILVER SULFADIAZINE AND HAIR FOLLICLE BULGE STEM CELLS CAN FACILATE WOUND HEALING IN RAT

fereshteh nejaddehbashi[1], mahmoud orazizadeh[2,3], mahmoud hashemitabar[4,3], vahid bayati[3,4], mohammadreza abbaspour[5], eskandar moghimipour[6]

[1] *Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

[2] *Department of Anatomical Sciences, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

[3] *Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[4] *Department of Anatomical Sciences, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[5] *Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran*

[6] *4.Nanotechnology Research center, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Corresponding author's email: fereshte_negad@yahoo.com

Despite special advances in regenerative medicine, wound healing is a challenging medical problem. The skin is the largest organ in the human body that has essential and important functions, so any damage to its normal structure should be treated as soon as possible. Easy access to skin stem cells has received a great deal of attention in its therapeutic applications. Cell therapy is a new method in restorative medicine, especially when old therapies have disappeared. Candidate populations for therapeutic applications include mesenchymal stem cells, hair follicle stem cells, and pluripotent cells. Hair follicle bulge stem cells with scaffold and growth factors can improve wound healing.

Materials and methods: In this study PCL/SSD, PCL/Coll as two -layered nanofibrous mat were designed with electrospinning. Bulge stem cells were isolated from rat vibrissa and cultured on two – layered nanofibrous mat with nanoparticles containing EGF and bFGF as growth factors. Nanoparticles containing EGF and bFGF were made with ionic gelation processes. EGF and bFGF release were analyzed with human EGF and bFGF Quntikine human ELIZA kit. Bulge stem cells were analyzed with Immunocytochemistry and flow cytometry. These cells were tracked with CM-Dill 7000C. Tracked cells were seeded on nanofibrous mat and grafted to rat skin. The healing process was evaluated with macroscopic observation on 7,14,21 and 28 days' post wounding. Hematoxylin - eosin and Trichromasons staining was done to evaluate tissue remodeling and collagen synthesis. Real time PCR analysis performed to assess gene expression after 14 days of treatment.

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Abstract **#1689**

THE BIOLOGICAL FUNCTIONS OF SMALL WORLD NEURONAL NETWORKS

Francesco Gentile[P][1]

[1] *University Magna Graecia of Catanzaro*

Corresponding author's email: francesco.gentile77@gmail.com

Neuronal cells are the smallest building blocks of the central and peripheral nervous systems. Information in neural networks and cell-substrate interactions have been heretofore studied separately. Understanding whether surface nanotopography can enhance the computational efficiency of neuronal networks may yield to decipher the brain's code, and provide new tools for a better understanding of neuro-degeneration and cell clustering. Here, we recapitulate recent studies of cell behavior at the region of contact between the cell with nanomaterials. Using functional, multi-calcium imaging techniques, and information theory approaches, these studies (1-4) have illustrated that for certain configurations of the nanomaterials and of the topography of the biointerface, neuronal cells form networks with small world characteristics. Small world networks feature high clustering and short paths between the cells of the networks: in small world networks, information is transported more efficiently than in equivalent random or periodic networks of the same size. Moreover, research also displayed that the maximum cluster size in those networks is of approximately 200 neurons. This value - resulting from the competition between the binding energy of cells with other cells and the substrate, and the kinetic energy of the system - is notably similar to the number found in the cortical minicolumns, i.e. the elementary computational units of the cerebral cortex. Results led us to formulate a biological principle of equivalence, i.e. the hypothesis that neurons evolve to form structures with a topology that minimizes energy and maximizes information.

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Abstract **#1690**

IN VIVO TRACKING OF THE IMMUNE RESPONSE TO ECM BIOSCAFFOLDS

Mike Modo[P][1]

[1] *University of Pittsburgh*

Corresponding author's email: mmm154@pitt.edu

Magnetic resonance imaging (MRI)-based guidance of extracellular matrix (ECM) hydrogel affords its implantation into a tissue cavity caused by a stroke. A major immune response occurs that leads to the biodegradation of the implanted hydrogel and a reconstitution of brain tissue ensues (2). To improve our understanding of the spatio-temporal dynamics of the immune cell infiltration into ECM hydrogel, we used 19F MR imaging to trace immune cells that were systemically labeled using perfluorcarbon (PFC) nanoemulsions. Using this approach the invasion of peripheral immune cells into peri-infarct stroke tissue was evident by 6 hours post-ECM implantation. Cell invasion into the ECM bioscaffold was observed 9 hours and a peak signal at 18 hours post-implantation. The 19F signal in the ECM hydrogel slightly decreased by 24 hours. Histological investigations of tissue revealed a strong infiltration of neutrophils into the ECM hydrogel at 24 hours, as well as macrophages. No lymphocytes were observed at this time point. Almost all neutrophils and macrophages contained PFC indicating that these were the source of the 19F MR signal. 19F MRI combined with PFC nano emulsions therefore provide a unique tool to assay the spatio-temporal response of immune cells to ECM hydrogel implantation.

Abstract **#1692**

DECIPHERING MOLECULAR EFFECTORS OF AMNIOTIC MEMBRANE HEALING EFFECTS ON CHRONIC WOUNDS USING A HUMAN TGF-SS-CHRONIFIED KERATINOCYTE CELL MODEL.

Francisco J. Nicolás[P1], Ángel Bernabé-García[1], Sergio Liarte[1], Mónica Rodríguez-Valiente[1,2], José María Moraleda[3,4], Gregorio Castellanos[3,2]

[1] Regeneration, Molecular Oncology and TGFß, IMIB-Arrixaca, Murcia, Spain

[2] Chronic wounds and Diabetic foot ulcer unit. IMIB-Arrixaca, Murcia, Spain

[3] Cell therapy unit, Regeneration, IMIB-Arrixaca, Murcia, Spain

[4] HEMATOLOGÍA. TRASPLANTE HEMATOPOYÉTICO Y TERAPIA CELULAR, University of Murcia, Spain

Corresponding author's email: franciscoj.nicolas2@carm.es

The application of Amniotic Membrane (AM) in chronic wounds has proven very successful at resetting wound healing, in particular re-epithelialization. TGF-ß plays a very important role at the wound healing process and alteration of its expression may end up in a chronification of the wound, thus impeding its natural healing. When applied on epithelial cells, AM activates several important signaling pathways required for migration and proliferation. Importantly, we have seen that AM exert a fine control on TGFß signaling pathway. To investigate AM molecular mechanisms behind its effect, we used cryopreserved AM on HaCaT´s wound healing scratch assay. In parallel, we measured the expression of important cell migration proteins at the wound healing front of HaCaT´s scratch assays. An important effect of AM in the cytoskeleton and focal adhesions restructuring was detected. The AM control of TGF-ß managed cell proliferation together with its stimulatory effect on cell migration could explain AM positive effect into wounds re-epithelialization. The chronic wound environment has been mimicked by long term TGFß-stimulation in the absence of serum of HaCaT cells. In this TGF ß chronified keratinocytes, the presence of AM produces a clear improvement of several issues related with successful wound healing. Amniotic membrane is a successful agent at healing complicated wounds, thus the precise knowledge of the molecular mechanisms involved on the phenomena will allow us to improve its application and to look for efficacious AM-based cell-based alternatives for future therapeutic application.

Abstract **#1693**

ANTI-INFLAMMATORY AND PRO-ANGIOGENIC ROLE OF AMNIOTIC MEMBRANE ON ENDOTHELIAL CELLS DERIVED FROM UMBILICAL CORD OF WOMEN AFFECTED BY GESTATIONAL DIABETES MELLITUS: NEW INSIGHTS IN DIABETIC FOOT ULCER.

Francisco J. Nicolás[P][1], Caterina Pipino[2], Ángel Bernabé-García[1], Pamela Di Tomo[2], Javier Stelling-Férez[3,1], Ilaria Cappellacci[2], Silvia Pappadà[2], Domitilla Mandatori[2], Letizia Pelusi[2], Assunta Pandolfi[2]

[1] Regeneration, Molecular Oncology and TGFß, IMIB-Arrixaca, Murcia, Spain

[2] Department of Medical, Oral and Biotechnological Sciences, Università G. d'Annunzio Chieti-Pescara, StemTeCh group, Chieti, Italy.

[3] Departamento de Nutrición y Tecnología de los Alimentos, UCAM, Murcia, Spain

Corresponding author's email: franciscoj.nicolas2@carm.es

Diabetes foot ulcer (DFU) is a severe diabetes complication which affects 15% of patients with advanced stages of the disease. Despite improvements in the treatment, DFU remains the main causes of amputation of the lower limb. Furthermore, one of the most relevant diabetic wounds healing issues is the reduced peripheral blood flow and the diminished neovascularization. Amniotic membrane (AM) has shown promising results in the treatment of DFU. Here, the potential role of AM on endothelial cells isolated from umbilical cords of gestational diabetes affected women (GD-HUVECs) have been investigated. Indeed, GD-HUVECs have shown reduced migration capacity and decreased vessels formation on Matrigel compared to control HUVECs, thus representing a useful model for studying the role of AM in the neovascularization of chronic non-healing wounds. Hence, GD-HUVECs cell migration and tube formation ability were evaluated in the presence of AM. Moreover, the anti-inflammatory properties of AM have been assessed using a monocyte-endothelium interaction assay stimulated with TNF-a. Results showed that AM treatment significantly improved cell migration and vessel formation in GD-HUVECs. Strikingly, AM significantly reduced TNF-a stimulated monocyte-endothelium interaction and the membrane exposure of the endothelial adhesion molecules VCAM-1 and ICAM 1 in C- and GD-HUVECs. These results suggest that AM improves chronic wounds healing by both enhancing angiogenesis and decreasing inflammation, thus reinforcing its clinical applications for diabetic foot ulcers. Additional preliminary data would be presented on the effect of oleanolic acid on the improvement of tubule formation assay on GD-HUVECs.

Abstract **#1694**

NATURAL TRITERPENOID OLEANOLIC ACID AND ITS COMPLEXATION WITH CYCLODEXTRINS: CELL MIGRATION EFFECTS AND FUTURE APPLICATIONS IN WOUND HEALING

Javier Stelling-Férez[P][1,2], Ángel García-Bernabé[1], David Armero-Barranco[3], Sergio Liarte[1], María Ruzafa-Martínez[3], Santiago López-Miranda[2], José Antonio Gabaldón[2], Francisco José Nicolás[1]

[1] *Regeneration, Molecular Oncology and TGFß, IMIB-Arrixaca, Murcia, Spain*

[2] *Nutrition and Food Technology Department, UCAM, Murcia, Spain*

[3] *Nursing Department, University of Murcia, Murcia, Spain*

Corresponding author's email: javier.stelling.ferez@gmail.com

Natural triterpenoids, particularly oleanolic acid (OA), have shown wound healing benefits. To understand OA effect on wounds, we have studied OA-triggered molecular mechanisms in two epithelial cell lines: Mv1Lu and MDA-MB-231, by using in vitro wound healing scratch assays. Besides these, we have studied pro-migratory proteins expression and its subcellular localization. Epithelial cells migration is essential for wound closure and rely on key regulator proteins. In this sense, we have shown that OA induces the activation, at cells of the wound edge, of c-Jun; a master regulator of migration. On top of that, cell migration is mediated by dynamic changes in cell architecture: cytoskeleton reorganization and focal adhesion (FA) remodeling. Thus, we have shown that OA activates focal adhesion kinase (FAK), a critical factor for FA remodeling, and also the reorganization of paxillin and actin, indicating that OA promotes a remodeling of the cell architecture. In addition, we have performed scratch assays in human fibroblasts, which are involved in tissue remodeling during wound healing. Similarly, OA enhances fibroblast migration. Due to its lipophilic nature, OA delivery to cells is difficult. Cyclodextrins are well-known to improve lipophilic molecule solubility and bioavailability, so we have created OA/cyclodextrin complexes to test them in migration assays. Strikingly, we have shown that these complexes facilitate OA in vitro use and enhance its cell migration properties. All these results encourage us to pursue on OA/cyclodextrins as a way of improving OA application in wounds.

Keywords

Oleanolic acid; Cyclodextrins; Cell migration

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Abstract **#1695**

X-RAY PHYSICS-BASED CT-TO-COMPOSITION CONVERSION APPLIED TO A TISSUE ENGINEERING SCAFFOLD, ENABLING MULTISCALE SIMULATION OF ITS ELASTIC BEHAVIOR

Karol Szlazak[P][1], Viktoria Vass[2], Patricia Hasslinger[2], Jakub Jaroszewicz[1], Alexander Dejaco[2], Joanna Idaszek[1], Stefan Scheiner[2], Christian Hellmich[2], Wojciech Swieszkowski[1]

[1] *Faculty of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland*

[2] *Institute for Mechanics of Materials and Structures, TU Wien – Vienna University of Technology, Vienna*

Corresponding author's email: karolszlazak@wp.pl

Nowadays, the assessment of the mechanical competence of tissue engineering scaffolds based on computer simulations is a well-accepted technology. Typically, such simulations are performed by means of the Finite Element (FE) method, with the underlying structural model being created based on micro-computed tomography (microCT). Here, this analysis modality is applied to a new, ternary composite, consisting of PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PLGA, poly(lactic-co-glycolide), and TCP, tricalcium phosphate hydrate. The studied scaffold structure is made up by fibers of this new composite material, manufactured by means of the rapid prototyping method. The data collected from microCT is utilized for adequately defining the mechanical properties of the FE model. In particular, the three-dimensional field of grey values is interpreted in terms of the underlying field of attenuation coefficients, taking into account the photon energy employed in microCT imaging. For the sake of keeping the FE simulations as efficient as possible, groups of voxels are combined into one finite element; the grey value of the latter is obtained by volume averaging. Employing a two-step micromechanical homogenization scheme, the experimentally accessible stiffness of the three constituents is then, finite element by finite element, upscaled to the composition-dependent stiffness of the composite material. The plausibility and adequacy of the FE model is demonstrated by simulating the effects of uniaxial compression on the scaffold structure, in terms of resulting stress and strain fields, highlighting the importance of the fiber junctions, and that neglecting the material heterogeneity would lead to a potentially significant underestimation of stresses and strains.

Keywords

microcomputed tomography; bone scaffolds; micromechanics

Abstract **#1697**

COMMERCIAL TRANSLATION FROM LAB TO TRADE SALE

Anthony S Weiss[P][1]

[1] *University of Sydney*

Corresponding author's email: *tony.weiss@sydney.edu.au*

We discovered how to assemble tropoelastin into synthetic elastin biomaterials that accelerate and improve the repair of scars and wounds. This work over two decades has led to a dominant position, and the patented biomaterials inventions recently led to one of the largest commercial transactions in regional healthcare history. The company completed in-depth primary market research which demonstrated the potential for bio-based TE products to address unmet, high-value market demand across multiple applications. The company also scaled production from lab to Good Manufacturing Practice, and successfully completed multiple clinical trials for injectables that enhance skin repair, including local damage and scars (1, 2, 3, 4). This strong position attracted a range of commercial suitors and the company was sold to a large biopharmaceutical company.

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Abstract **#1698**

BUILDING AND REPAIRING ELASTIC TISSUES

Anthony S Weiss[P][1]

[1] *University of Sydney*

Corresponding author's email: tony.weiss@sydney.edu.au

Elastic fibers are integral components of the resilient extracellular matrix. They provide the structural support and elastic recoil required for the continuous mechanical stretching and recovery of soft force-bearing tissues with durability and persistence. They are made using a protein intermediate that possesses a structure that is organized enough for assembly but flexible enough to confer elasticity. This tightly controlled hierarchical process delivers versatile materials that are used for in vitro building of three dimensional elastic materials, in vivo tissue assembly and repair, and unique hybrid materials. Elastin's properties have made it an attractive candidate for incorporation into biomaterials for a range of applications in regenerative medicine. This presentation will summarize recent advances in understanding how the sequence and structure of elastin influence the processes that govern the functionality of elastin and its derivatives, and describe innovations in harnessing these mechanisms to create tunable elastin-based biomaterials (1, 2, 3, 4, 5).

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Abstract **#1699**

A NOVEL APPROACH TO HEPATOCELLULAR CARCINOMA: EFFECTS OF PROTEIN FRACTIONATION, THERMAL DENATURATION AND LIPIDS ISOLATION FROM A HUMAN AMNIOTIC MEMBRANE EXTRACT

Ricardo Teixop[1,2,3,4], Mafalda Laranjo[1,2,3,4], Maria João Carvalho[1,2,3,4,5], Beatriz Serambeque[1,2,3], Patrícia de Oliveira[1,2,3], José Paulo Moura[5], Ana Margarida Abrantes[1,2,3,4], Pedro Domingues[6], Maria Filomena Botelho[1,2,3,4]

[1] *1.Biophysics Institute, Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

[2] *2.Institute for Clinical and Biomedical Research (iCBR) area of Environmental Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

[3] *3.Clinical Academic Center of Coimbra, Coimbra, Portugal*

[4] *4.Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal*

[5] *5.Gynecology Service, Coimbra Hospital and University Centre, Coimbra, Portugal*

[6] *6.Centro de Espetrometria de Massa, Departamento de Química & QOPNA& LAQV-REQUIMTE, University of Aveiro, Aveiro, Portugal*

Corresponding author's email: ricardo.teixo@gmail.com

Introduction: Hepatocellular carcinoma (HCC) is most common liver cancer and presents poor prognosis. Human amniotic membrane (hAM) has interesting anti-tumor properties. We previously showed that hAM extract (hAME) leads to anti-tumor effects on HCC. We aimed to assess effects of hAME fractions (protein/lipidic) and thermal denaturation on HCC cells.

Methods: Fractionation was performed through ammonium sulphate (AS) precipitation by sequentially adding 10%, 25% and 50% AS to hAME, on ice, 15min, centrifuged at 14000 G, 15 min. Precipitated fractions (10P/25P/50P) were resuspended on PBS; soluble fractions (10S/25S/50S) were submitted to salting out with PBS by centrifugation (4000G, 60 min), on VivaSpin® tubes (30 kDa cutoff). Lipid isolation was performed by Folch method (chloroform:methanol (2:1), two extraction steps and solvent evaporation on vacuum). hAME thermal denaturation was performed at 100ºC, 5min. HepG2, Hep3B and HuH7.sil cells were incubated with total hAME, fractions, thermal denaturated hAME and isolated lipids (1µg/µL), for 72h. Metabolic activity was accessed by MTT assay.

Results: Results showed decreased metabolic activity of HCC cells incubated with hAME, compared to control (HepG2: 57.07±9.26; Hep3B: 50.66±9.78; HuH7.sil: 58.16±6.05). Protein fractions induced a lower metabolic activity (2 to 30%, cell line-dependent) compared to total hAME. Incubation with isolated lipids decreased metabolic activity of HCC cells, compared to control and total hAME. Thermal denaturation partially inhibited effects of total hAME.

Conclusions: These findings indicate that both proteins and lipids could have an important role on hAME induced anti-tumor activity.

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Abstract **#1700**

TREATMENT OF LIVER DISEASES, VENTURING BEYOND ORGAN AND CELL TRANSPLANTATION

Robert Anthony Fisher P[1]

[1] *CTI Clinical Trial & Consulting*

Corresponding author's email: rafishertransplant@gmail.com

There has been fifty years of thoughtful in vitro and in vivo animal modeling together with optimized hepatocyte isolation, banking and transformational stem cell and genomic cell editing; combined with thirty years of clinical hepatocyte transplantation. The lessons learned from more than 72 brave hepatocyte transplanted human souls have provided multiple proofs of the concept of "back to the basic" hepatocyte treatment for human liver disease that instructs disruptive developing cell editing combined with liver matrix biology.(1) Cellular transplant of human liver disease as a standard of care requires an available hepatocyte supply with standardized quality and function parameters; combined with standardized, effective, safe proliferation, engraftment techniques with optimal transplanted cell tracking and standard clinical outcomes registry.(2) The metabolic liver disease optimal treatment will be disrupted by the use of a safe small fraction of the patients own hepatocytes with direct correction of the mutation using CRISPR (clustered, regularly interspaced, short palindromic repeats) technology, tested with target cell humanized chimeric experimental animals with a limited preconditioning protocol of the transplant recipient.(3) In addition the non hereditary metabolic liver affected patient may disrupt the inadequate liver supply dilemma with use of their own fibroblasts to be induced into pluripotent stem cells and then reprogrammed into fully functional expandable hepatocytes for auto-transplant.(4,5) The perfect storm for innovation includes the hepatocyte supply fix above, combined with the transformational technology to decellularize human liver as the optimal scaffold for liver creation, transplantation nidation and regenerative study.(6,7)

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Abstract **#1701**

ULTRATHIN HYDROGEL COATINGS FOR FUNCTIONALIZATION OF GLASS AND TISSUE MODELLING APPLICATIONS

Vytautas Cepla[1], Tomas Rakickas[1], Gintarė Stankevičienė[1], Airina Mazėtytė-Godienė[1], Ramūnas Valiokas[1]
[1] *Department of Nanoengineering, Center for Physical Sciences and Technology, LT-02300 Vilnius, Lithuania*
Corresponding author's email: vytautas.cepla@ftmc.lt

We have studied initiator free, UV-controlled self-initiated photografting and photopolymerization reaction on glass substrates. By combining surface chemical modifications and 2-hydroxyethyl methacrylate, PEG methacrylate and methacrylic acid monomers we determined the conditions for efficient synthesis that we monitored by employing a set of surface analysis techniques. Furthermore, we studied the swelling behaviour of the resulting hydrogel coatings (HPMAA) [1]. Also, we microfabricated and characterized fibronectin (FN) patterns on such coatings aiming at efficient mimetics of the extracellular matrix. The resulting glass-supported HPMAA layers performed as reliable substrates for microfabrication of covalently bound FN patterns for controlled cell adhesion. The obtained FN patterns consisted of densely packed protein molecules. They maintained the structural integrity upon HPMAA swelling. Finally, we evaluated the applicability of the synthesized HPMAA hydrogel coatings as substrates for fabrication of tissue-on-a-chip modelling systems using human corneal epithelial cells and human skin fibroblasts. Our study paves the way to new solutions toward efficient tissue mimetics and engineering that are compatible with the state-of-the-art microscopy techniques.

Keywords
Ultrathin hydrogel coating ; protein patterns; tissue model

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Abstract **#1702**

MESENCHYMAL STEM CELL-DERIVED SMALL EXTRACELLULAR VESICLES PROMOTE BONE REGENERATION THROUGH A MULTI-FACETED MECHANISM OF ACTION

Wei Seong Toh[1,2,3], Chee Weng Yong[1], Shang Jiunn Chuah[1], Raymond Wong[1], Asher Lim[1], Sai Kiang Lim[4,5]

[1] *Faculty of Dentistry, National University of Singapore, Singapore*

[2] *Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

[3] *Department of Biomedical Engineering, Faculty of Engineering, National University of Singapore, Singapore*

[4] *Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore*

[5] *Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

Corresponding author's email: dentohws@nus.edu.sg

Mesenchymal stromal/stem cells (MSCs) have reported therapeutic efficacy for bone repair in animal and clinical studies. Although the therapeutic effects of MSCs were originally predicated on the differentiation potential of these cells, it is now widely accepted that MSCs mediate tissue repair through the secretion of trophic factors, particularly 50-200nm small extracellular vesicles (sEVs) which include exosomes. In this study, we investigated the therapeutic effects of MSC-sEVs loaded in collagen sponge for regeneration of critical-size bone defects in an immunocompetent rat calvaria defect model. We observed that sEV-mediated repair of critical-size bone defects was characterized by increased cellular proliferation and infiltration, enhanced vascularization and mineralization, and a regenerative immune phenotype. Specifically, defects treated with MSC-sEVs displayed a regenerative immune phenotype characterized by a higher infiltration of CD206+ regenerative M2 macrophages over CD86+ pro-inflammatory M1 macrophages, with a concomitant reduction in pro-inflammatory IL-1β+ and TNF-α+ cells. To gain insights to the cellular processes activated by MSC-sEVs during bone repair, cell culture studies utilizing MSCs, endothelial cells and macrophages were performed. We observed that addition of MSC-sEVs accelerated osteogenic mineralization of MSCs and promoted angiogenic tube formation of endothelial cells in a dose-dependent manner. Furthermore, addition of MSC-sEVs to macrophages enhanced M2 macrophage polarization while suppressing M1 macrophage polarization. Together, these findings demonstrated that MSC-sEVs work through a multi-faceted mechanism of action to promote bone regeneration.

Keywords

Mesenchymal stem cells; Extracellular vesicles; Bone regeneration

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Tan SHS et al. Mater Today Bio. 7, 100067, 2020

Abstract **#1703**

NON-INVASIVE STEM CELL THERAPY. REVISITING SHOCK WAVE THERAPY. NEW BASIC SCIENCE AND NEW CLINICAL APPLICATIONS.

Daniel Schlatterer Schlatterer[P][1], John Keating[1], Ken Lippe[1], Derrick Wendler[1]

[1] *Wellstar Atlanta Medical Center*

Corresponding author's email: danschlatterer67@gmail.com

Numerous reports in the literature demonstrate reparative benefits of stem cells. Obstacles to widespread clinical stem cell usage include regulatory and sourcing of stem cells. Moreover, harvesting, processing and application of stem cells have inconsistencies. Overcoming many of these challenges could be achieved if a mechanism to stimulate in vivo stem cell release were developed. In the mid 1980's, shock wave therapy(SWT) was invented in Germany to treat gallstones noninvasively. SWT soon found application for kidney stones as well, commonly referred to today as lithotripsy. Over the past 35 years, the clinical indications for SWT have expanded. The purpose of this paper is to present the SWT stem cell basic science data that has been obtained and to demonstrate clinical examples of soft tissue success with SWT in an orthopaedic practice

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Abstract **#1704**

ANTI-BIOFILM THERAPIES IN DENTISTRY

Hamdan Alghamdi[P][1]

[1] *Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, Riyadh, Saudi Arabia*

Corresponding author's email: dalghamdi@ksu.edu.sa

In fact, oral diseases are the collective effect of the bacteria community in the dental biofilm. Nutritional synergy between bacteria can be seen in the development of dental biofilm. Dental biofilms are well implicated in dental/oral chronic infections. Biofilms are multicellular communities of microbial organisms embedded in extracellular polymeric matrix that forms the 3D scaffold of biofilm and is responsible for adhesion to surfaces. A cell–cell communication mechanism, called quorum sensing (QS), plays an important role in biofilm formation. The microbial organisms could disperse from the biofilm to find new niches and initiate new biofilm formation (i.e. biofilm life cycle).

Therefore, it is still demanding to discover new therapies that can dysregulate biofilm formation and development. The strategies of anti-biofilm therapies should target on each step of biofilm development, including the inhibition of microbial attachment, disturbing polymeric matrix generation, and interruption of QS signaling.

Recently, several therapeutic strategies have been proposed to combat dental biofilms. One is the inhibition of bacterial adhesion to dental surfaces. Therefore, it is important to consider the physical structural organization of multispecies biofilm from physical point of view.

Second, extensive efforts have been made to develop dental biomaterials with control-releasing antimicrobial property.

This presentation will summarize recent scientific knowledge on the recent ingredients and substances with clear-cut anti-biofilm properties. Besides, anti-biofilm therapeutic strategies undergoing clinical trials are highlighted.

Abstract **#1705**

3D PRINTING OF NANOCOMPOSITE HYDROGELS/POLYMERS FOR LOCAL DRUG DELIVERY APPLICATIONS

Nermin Seda Kehr$_P$[1]

[1] *WWU Münster*

Corresponding author's email: seda@uni-muenster.de

Injectable hydrogels/polymers have received considerable interest in the biomedical field due to their potential applications in minimally invasive local drug delivery, more precise implantation, and site-specific drug delivery into poorly reachable tissue sites and into interface tissues, where wound healing takes a long time.[1] Furthermore, injectable hydrogels/polymers are important for improving the efficiency of cancer treatment: They can provide site-specific delivery of chemotherapeutics into cancer tissue and, thus, limit the potential circulation of the chemotherapeutics in the body, thereby reducing the toxic side effects of chemotherapeutics to healthy tissues.[2]
In this context, we described injectable hydrogels/polymers made of stimuli responsive nanomaterials.[2,3] The prepared biomaterials were used to generate multifunctional 3D NC scaffolds via by 3D printing technique and used for cell- or bacteria-biomaterial interaction studies. Our results showed that the 3D printed NC scaffolds provide sustained and pH-responsive release of drug molecules that improved cell adhesion and inhibited bacterial growth on the NC scaffolds surfaces.[3] Furthermore, 3D NC scaffolds inhibit the growth of cancer cells, while improve the viability of healthy cells.[2]

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Abstract **#1706**

INTRA-ARTICULAR DELIVERY OF CARTILAGE- AND SYNOVIOCYTE-BINDING NANOCOMPOSITE MICROGELS FOR OSTEOARTHRITIS TREATMENT

Lina Maria Mancipe Castro[P][1,2,3], Abigail Sequeira[4], Michael Hunckler[1,2], Andres J. Garcia[1,2], Robert E. Guldberg[3]

[1] *Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology 315 Ferst Dr NW, Atlanta, GA 30332, U.S.A.*

[2] *George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology 315 Ferst Dr NW, Atlanta, GA 30332, U.S.A.*

[3] *Phil and Penny Knight Campus for Accelerating Scientific Impact, University of Oregon 6231 University of Oregon, Eugene, OR 97403-6231*

[4] *School of Chemical and Biomolecular Engineering, Georgia Institute of Technology 311 Ferst Drive NW, Atlanta, GA 30332, U.S.A.*

Corresponding author's email: linamc@uoregon.edu

Intra-articular (IA) injection is an attractive route of administration for the treatment of osteoarthritis (OA). However, free drugs injected into the joint space are subjected to clearance mechanisms, which reduce their IA retention time. Additionally, several drug candidates can induce adverse off-target effects on different IA tissues. To overcome these limitations, here we propose the use of tissue-binding, nano-composite microgels as IA small molecule drug delivery vehicles. Micron-scale poly(ethylene glycol) (PEG) hydrogels, presenting cartilage- or synoviocyte-binding peptides and containing small molecule-loaded poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs) were synthesized using microfluidics technology. Microgels loaded with a model small molecule (rhodamine B) exhibited a sustained, near-zero order release over 16 days. Additionally, PEG microgels conjugated with synoviocyte- or cartilage-targeting peptides, presented specific binding to rabbit and human synoviocytes, and to bovine articular cartilage in vitro, respectively. Using a rat model of knee OA, microgels were shown to be retained in the IA space for at least 3 weeks and did not induce detectable joint degenerative changes as measured by EPIC-µCT and histology. Finally, histological analysis demonstrated that all microgel formulations get trapped within the synovial membrane and significantly increase the IA retention time of a model small molecule near infra-red dye in vivo. Overall, these results suggest that nano-composite PEG microgels presenting tissue-binding peptides could be a promising strategy to achieve tissue-localized drug delivery and prolonged IA retention of small molecule drugs for OA treatment.

Keywords

Osteoarthritis; Microgels; Tissue-binding

Abstract **#1708**

EMBEDDING FLEXIBLE BIOELECTRONICS WITHIN 3D SCAFFOLDS FOR CELLULAR INTERROGATION AND CARDIAC TISSUE ENGINEERING.

Dannielle Cox-Pridmore[1,2], Yunlong Zhao[1,2], Fernando Castro[1], Patrizia Camelliti[2], Ravi Silva[2]

[1] *National Physical Laboratory*

[2] *University of Surrey*

Corresponding author's email: dannielle.cox-pridmore@npl.co.uk

Cardiac tissue engineering (CTE) is the in vitro creation of functional heart tissue that can elucidate tissue development, disease progression, pharmaceutical efficacy, or be implanted to replace lost heart functioning [1, 2]. Three-dimensional (3D) scaffolds are often used as a template for cardiomyocytes (heart muscle cells) to form cardiac tissue. However, more active supports may be required as current methods are unable to engineer tissue that is representative of endogenous myocardium [3, 4].

In this project, flexible, porous, biocompatible micro-bioelectronic meshes were combined within a traditional CTE arrangement of hydrogel and cardiomyocytes. Embedding the mesh allows for the seamless incorporation of electronic components within the engineered cardiac tissue. Such components include flexible electrodes and the provision of electrical stimulation (ES), which is a proven method of influencing cell alignment, contractility, and maturation [5]. While integrated biosensors can achieve extensive monitoring by detecting cell impedance and electrophysiology, allowing for a valuable understanding of the tissue being created [6, 7].

The biomimetic design of the electronic devices allows for the efficient diffusion of oxygen, nutrients, and metabolic waste as well as the movement and penetration of heart cells for more reliable cardiac tissue growth [8]. The introduction of flexible mesh bioelectronics within CTE methods allows for an intimate interaction with cardiac cells, monitoring capabilities, and the regulation of tissue formation. Such additions could overcome current limitations and produce physiologically relevant cardiac tissue that has the potential to be used in both research and implantation.

Keywords

Cardiac Tissue Engineering; Electrical Stimulation ; Biosensors

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Abstract **#1709**

CELLULAR CROSS-TALK-INDUCED SECRETION OF INTERLEUKIN-10 (IL-10) IN AN ORGANOTYPIC HUMAN MELANOMA-IN-SKIN MODEL DIRECTS MONOCYTE DIFFERENTIATION TOWARDS AN M2-LIKE PHENOTYPE

Elisabetta Michielon[P][1], Marta López González[2], Judith L.A. Burm[1], Taco Waaijman[1], Ekaterina S. Jordanova[3], Tanja D. de Gruijl[2], Susan Gibbs[1,4]

[1] Department of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit, Amsterdam, The Netherlands

[2] Department of Medical Oncology, Amsterdam UMC, Vrije Universiteit, Cancer Center Amsterdam, Amsterdam Infection & Immunity Institute, Amsterdam, The Netherlands

[3] Center for Gynecologic Oncology Amsterdam (CGOA), Amsterdam UMC, Vrije Universiteit, Amsterdam, The Netherlands

[4] Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit, Amsterdam, The Netherlands

Corresponding author's email: e.michielon@amsterdamumc.nl

Preclinical assessment of novel therapies to fight cancer requires models that reflect the human physiology and immune response. While two-dimensional (2D) cultures lack tissue context, animal models poorly predict human immune responses, with the result that potential new drugs fail in the clinical setting. Here, we established an in vitro three-dimensional (3D) reconstructed organotypic human melanoma-in-skin (Mel-RhS) model to investigate tumor formation and progression over a six week period. Tumor nests were observed to develop over time and to spread towards the dermis, disrupting the basement membrane. This coincided with the secretion of matrix metalloprotease 9 (MMP-9) by melanoma cells. These features resemble the initial stages of invasive melanoma. Interestingly, while interleukin-10 (IL-10) could not be detected in monolayers of the employed SK-MEL-28 melanoma cell line, the cellular cross-talk in the 3D model led to IL-10 expression by the melanoma cells, as well as by the surrounding keratinocytes and fibroblasts. Indeed, culture supernatants from Mel-RhS interfered with monocyte-to-dendritic-cell differentiation, leading to the development of M2-like macrophages, which was partly prevented upon antibody-mediated IL-10 blockade. Thus, the 3D configuration of the Mel-RhS model revealed a role for IL-10 in immune escape through misdirected myeloid differentiation, demonstrating the potential of the Mel-RhS in research on melanoma development and invasion in the setting of an immune competent model.

Keywords

IL-10; M2 macrophages; melanoma

Abstract **#1710**

LIPID VECTORS FOR THE DELIVERY OF CMRNAS WITHIN A BIOMATERIAL MATRIX FOR BONE REGENERATION

Elise Bonvinp[1], Cédric Sapet[1], Albane Birault[1], Florent Poulhes[1], Yolanda Sanchez-Antequera[2], Christian Plank[2], Olivier Zelphati[1]

[1] *OZBIOSCIENCES*

[2] *ETHRIS GMBH*

Corresponding author's email: ebonvin@ozbiosciences.com

Chemically modified messenger RNA-Lipid nanoparticles (cmRNA-LNP) represents a powerful tool not only for vaccine applications but also in regenerative medicine. Gene Activated Matrices (GAM), consisting in biomaterial formulations containing cmRNA-vector have been proposed to serve as support to transfect the colonizing cells and ensure localization of the cmRNA in the tissue to be regenerated. Despite the RNA chemical modification increases its in vivo stability, vectors or delivery vehicles are still required for efficient transfection and optimal pharmacokinetic in vivo. Thus, nanomaterials that can protect efficiently cmRNA and facilitate its uptake would be of great help in regenerative therapy. The goal of the project is to design, screen, study and select a lipid-based transfection reagent that will represent the best vector for cmRNA delivery in vivo. For this purpose, Ethris has designed and developed stable and efficient cmRNAs while OZB has screened a library of lipid-based transfection reagents for their capacity to complex cmRNA, protect and release it within hydrogels. A screening was performed by mixing cmRNA with the different novel synthesized lipids while LNPs were formed using a microfluidics technology. The five top reagents have been extensively characterized for their physicochemical properties (size, charge), complexation, release capacities and transfection efficiency. We show that high quality cmRNA together with our best candidates offer a highly relevant system for GAM-mediated hMSCs transfection. Their capacity to modulate vascularization, innervation and osteogenesis in vivo will be further studied.

Keywords

gene-activated matrix; cmRNA; transfection

Abstract **#1711**

BURSA-DERIVED CELLS RESPOND DIFFERENTLY TO PHYSIOLOGICAL AND PATHOLOGICAL MECHANICAL LOADING

Franka Klatte-Schulz[P][1,2], Nicole Bormann[1,2], Isabel Voss[1], Josephine Melzer[1], Aysha Schmock[1,2], Christian H. Bucher[1,2], Kathi Thiele[3], Philipp Moroder[3], Melanie Haffner-Luntzer[4], Anita Ignatius[4], Georg N. Duda[1,2], Britt Wildemann[1,5]

[1] *Julius Wolff Institute, Charité-Universitätsmedizin Berlin*

[2] *BIH-Center for Regenerative Therapies, Charité-Universitätsmedizin Berlin*

[3] *Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin*

[4] *Institute of Orthopaedic Research and Biomechanics, Ulm University*

[5] *Experimental Trauma Surgery, Department of Trauma-, Hand- and Reconstructive Surgery, University Hospital Jena*

Corresponding author's email: franka.klatte@charite.de

The subacromial bursa is an important friction-reducing structure in the shoulder joint, but knowledge regarding the mechanical competence of bursa tissue is missing. Therefore, the study aimed to investigate how bursa-derived cells respond to physiological and pathological loading regimes.

Bursa-derived cells were stimulated over 3 days for 1h or 4h with 1%, 5%, or 10% strain on collagen-coated silicon dishes. Orientation of actin cytoskeleton, YAP nuclear translocation and activation of non-muscle myosin II (NMM-II) was evaluated for 4h-stimulations to investigate mechano-transduction processes. After 1 and 4h, cell viability, gene expression of Integrin A1/A2, Collagen I/III, Versican, Fibromodulin, MMP1/2/3, TIMP1/2 and protein secretions were analyzed.

With higher loading, the orientation angle of the actin cytoskeleton significantly increased towards a perpendicular direction. The lowest variations in orientation occurred for the 5% loading group. With pathological loading (10%), cells showed a loss in actin density and a slightly reduced cell viability. A significantly increased YAP nuclear translocation was observed for the 1% loading group with a similar trend for the 5% group. NMM-II activation was weak for all conditions. On gene expression level, only the TIMP2 expression was significantly decreased in the 1h group compared to control. Collagen I and MMP2 protein secretion significantly increased with pathological loading, whereas TIMP1 secretion was significantly reduced leading to an MMP/TIMP imbalance.

This study documents for the first time a clear mechano-responsiveness of bursa-derived cells with specific reactions to physiological and pathological loading conditions. This hints to a physiological function of mechanical loading in bursa-derived cells.

Keywords

Bursa-derived cells; Mechanical stimulation; Mechano-transduction

Abstract **#1712**

AN IMMUNO-MODULATORY DEVICE FOR REDUCING POST-SURGICAL INFLAMMATION AND FIBROSIS IN GLAUCOMA

Mark Lemoine$_P$[1,2,3], Zülal Demir[1,2,3], Colm O'Brien[4,5], Fergal O'Brien[1,2,3], Alan Hibbitts[1,2,3]

[1] *Tissue Engineering Research Group, Dept. of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland.*

[2] *Trinity Centre for Biomedical Engineering, Trinity College Dublin, The University of Dublin, Dublin, Ireland.*

[3] *Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland.*

[4] *Dept of Anatomy, University College Dublin, Dublin, Ireland.*

[5] *Institute of Ophthalmology, Mater Misericordiae University Hospital Dublin, Dublin, Ireland.*

Corresponding author's email: marklemoine@rcsi.ie

Drainage tube or trabeculectomy surgeries in late-stage glaucoma remains troubled by post-operative inflammation and fibrosis. Current approaches to remedy this rely on administration of anti-metabolites (mitomycin C) and an intensive course of patient-administered corticosteroid drops. These present their own issues, particularly adverse side-effects and poor patient compliance. Therefore, we describe a proteoglycan (PG)-modified hyaluronic acid (HyA) drug-device that aims to address post-surgical inflammation and remove patient compliance issues.Hydrogels were assessed for crosslinking, swelling, degradation and insertion into cadaveric rabbit eyes. The innate ability of the modified gels to reduce collagen deposition in the absence of drugs was tested using primary human conjunctival fibroblasts stimulated with Transforming Growth Factor-Beta (TGF-β). The device was further enhanced using prednisolone drug loading with release assessed over 28 days and efficacy evaluated in a chick embryo model.Hydrogels were capable of rapid swelling, stability up to 4 weeks and enzymatic degradation. The device was easily implanted using standard surgical tools. In vitro analysis using drug-free, PG-HyA gels in TGF-β stimulated conjunctival fibroblasts demonstrated a reduction to normal collagen levels at 24 hours. Release of prednisolone from gel composites were found to exhibit a biphasic release with burst release over 72 hours and sustained release up to 28 days. Furthermore, this was found to effectively inhibit angiogenesis at 5 days ex-vivo. This work demonstrates proof of concept validation for a straightforward solution to a long running issue in glaucoma. Work now focuses on pre-clinical studies.Supported by Science Foundation Ireland:17/TIDA/5098. Enterprise Ireland: CF-2019-0764Y and CF-2020-1336

Abstract **#1713**

3D PRINTED SCAFFOLD COMBINED TO 2D OSTEOINDUCTIVE COATINGS TO REPAIR A CRITICAL-SIZE MANDIBULAR BONE DEFECT

Charlotte Garot[P][1,2], Michael Bouyer[1,2,3,4], Paul Machillot[1,2], Julien Vollaire[5,3], Vincent Fitzpatrick[2], Sanela Morand[1,2,6], Jean Boutonnat[7,8], Véronique Josserand[5,3], Georges Bettega[5,6], Catherine Picart[1,2]

[1] *CEA, CNRS, Université de Grenoble Alpes, ERL5000 BRM, IRIG Institute, 17 rue des Martyrs, F-38054, Grenoble, France*

[2] *CNRS and Grenoble Institute of Engineering, UMR5628, LMGP, 3 parvis Louis Néel, F-38016 Grenoble, France*

[3] *Université Grenoble Alpes, Institut Albert Bonniot, F-38000 Grenoble, France*

[4] *Clinique Générale d'Annecy, 4 chemin de la tour la reine –74000 Annecy, France*

[5] *INSERM U1209, Institut Albert Bonniot, F-38000 Grenoble, France*

[6] *Service de chirurgie maxillo-faciale, Centre Hospitalier Annecy Genevois, 1 avenue de l'hôpital –74370 Epagny Metz-Tessy, France*

[7] *Unité médico-technique d'Histologie Cytologie expérimentale, Faculté de Médecine, Université Joseph Fourier, 38700 La Tronche, France*

[8] *Département d'Anatomie et Cytologie Pathologique, Institut de biologie et de pathologie, Centre Hospitalier Universitaire de Grenoble, France*

Corresponding author's email: charlotte.garot@cea.fr

The reconstruction of large bone defects (> 5 cm3) remains a challenge for clinicians. To address this challenge, we developed a new critical-size mandibular bone defect model on mini-pigs (12 cm3), close to human clinical issues. We analyzed the bone reconstruction obtained from a 3D-printed scaffold made of clinical-grade PLA, coated with a polyelectrolyte film delivering an osteogenic bioactive molecule (BMP-2) at different doses (20, 50 and 110 µg/cm3 of scaffold, corresponding to 0.02-0.08 mg/mL). We compared the results of new bone quality and quantity using CT-scans, µCT, and histology to what is obtained with the gold standard solution, bone autograft. We proved that the dose of BMP-2 delivered from the scaffold through the polyelectrolyte film significantly influenced the amount of regenerated bone, its mineralization, and the repair kinetics, with a clear BMP-2 dose-dependence. Furthermore, bone was homogeneously formed inside the scaffold with little ectopic bone formation, independently of the BMP-2 dose used. Bone repair was as good as for the bone autograft. The BMP-2 doses applied in our study were reduced 20 to 75-fold compared to the commercial collagen sponges currently used in the clinical applications (dose of 1.5 mg/mL), without any adverse effects. 3D-printed PLA scaffolds combined with 2D biomimetic polyelectrolyte films and loaded with reduced doses of BMP-2 thus open perspectives in personalized medicine, since 3D-printing allows the customization of the scaffolds shape and the biomimetic film allows the controlled delivery of BMP-2 in space and time.

Abstract **#1714**

ENGINEERING A NOVEL IN VITRO MODEL OF THE BLOOD BRAIN BARRIER

Christina Schofield$_P$[1], Aleixandre Rodrigo-Navarro[1], Tom van Agtmael[2], Matt Dalby[1], Manuel Salmeron-Sanchez[1]
[1] *Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, UK*
[2] *Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK*
Corresponding author's email: c.schofield.1@research.gla.ac.uk

The blood-brain barrier (BBB) is a dynamic interface, regulating the movement of solutes, for example metabolites and drugs, between blood and brain. While the barrier is predominantly formed by the endothelial cells (ECs), these characteristics are not intrinsic to the cells and are induced by the relationships between the different cells which exist within the neurovascular unit (NVU). [1] Neither static nor dynamic in vitro BBB models fully capture in vivo-like conditions, and while coculturing EC monolayers with other NVU cell types has induced better barrier properties, the complexity of these culture conditions detracts from their usefulness for high-throughput drug discovery, testing, and disease modelling. Our proposed model utilises material-driven fibrillogenesis by PEA to present an EC monolayer grown on an electrospun PLLA membrane, which promotes the synergy of growth factor and integrin binding sites available on fibronectin (FN). While there are a handful of papers on electrospun-based BBB in vitro models [2] [3], we hypothesise that an effective in vitro model can be created with the use of key growth factors and EC monolayer grown on ECM protein coated PEA/PLLA electrospun membranes, which additionally provide a more flexible, biodegradable, and potentially thinner scaffold on which the ECs can grow.
We have demonstrated that this is a versatile, tuneable design capable of inducing barrier characteristics in both hiPSC and immortalised brain microvascular cells and have been able to show that different growth factor combinations, as well as protein coatings in combination with FN, are all capable of being incorporated.

Keywords
Blood brain barrier; In vitro model; Electrospinning

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Abstract **#1715**

HYBRID GLYCO-BIOMATERIALS ABLE TO MIMIC THE ECM MICROENVIRONMENT FOR 3D BIOPRINTED IN VITRO MODELS

Federica Barbugian$_P$[1]

[1] *Università degli studi di Milano Bicocca - Dipartimento di Biotecnologie e Bioscienze*

Corresponding author's email: f.barbugian@campus.unimib.it

Glycans are involved on cell fate modulation in physiological and pathological conditions. The intricate glycosignature at cellular and extracellular level is strongly involved in cell-cell and cell-ECM interactions that are at the basis of cell adhesion, spreading, differentiation and proliferation events [1]. The generation of glycoconjugate biopolymers to mimic ECM composition are a fascinating way to obtain tailorable in vitro systems able to replicate the cell microenvironment [2].
In this contest hyaluronic acid (HA) – a polysaccharide ubiquitous in ECM - represent a versatile platform for the development of tissue engineering and 3D advanced in vitro systems applications.
These different application perspectives are based on the possibility to fine-tune the physiochemical properties of the HA-based hydrogel by adjusting the concentration and degree of functionalization, resulting in tailored gelling behavior, viscosity, elasticity, mechanical strength, and also bioresponsiveness within biological systems. Here in this work, HA has been employed by conjugation with different ECM proteins taking advantage of linkers with different length and branching. The final goal of the project is to understand and test the biochemical and physical behavior of these different combinations in order to obtain a bioink as similar as possible to the ECM. Since we want to use these models for the study of brain tumor development, high-performance predictive screening, and the development of more effective drug therapies also their biocompatibility will be evaluated.

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Abstract **#1716**

NANOSTRUCTURED SCAFFOLDS MIMICKING FUNCTIONAL MENISCUS EXTRACELLULAR MATRIX (ECM)

Federica Barbugian$_P$[1]

[1] *Università degli studi di Milano Bicocca - Dipartimento di Biotecnologie e Bioscienze*

Corresponding author's email: f.barbugian@campus.unimib.it

Meniscus injuries are the most common knee injury among athletes [1]. Despite many advances in orthopedic sports medicine, emerging data suggest that fewer and fewer athletes are able to return to sport at the same level as before their injury. In fact, many of them continue to develop arthrosis at a younger age.

With the present study we want to produce a living construct based on 3D bioprinted fibrous scaffolds, designed ad hoc for the patient, that can be implanted and integrated in damaged site for meniscal replacement.

In order to ensure a better re-vascularization, tissue remodeling and healing, one of the aspects to be taken into account for grafting and faster post-operative recovery is also the cellular component. For this reason, nanostructured will be loaded with patient-derived mesenchymal stromal cells (MSCs) [2].

The produced scaffolds will be obtained by functionalization and adsorption with ECM derived growth factors (GF) and bioadhesive motifs to facilitate pre-vascularization with different degrees of functionalization and release.

The ultimate goal of the project is to provide a construct (cellular and acellular constructs) that are able to recapitulate the full biomechanical properties, biological characteristics to replace the loss of native tissue. The final implantable construct will also have a degradation rate that allows proper regeneration of the structure and induce a chondro-protective effect [3].

Keywords

Scaffolds; Meniscus; 3D printing

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Abstract **#1717**

CHARACTERIZATION OF IMMUNE BEHAVIOR/IMPACT IN A DEVELOPMENTAL 3-D BIOPRINTED RPE/CHOROID MODEL

Russ Quinn[P][1], Min Jae Song[2], Eric Nguyen[1], Anju Singh[3], Rafael Villasmil[4], Ty Voss[2], Tea Soon Park[1], Devika Bose[1], Marc Ferrer[2], Kapil Bharti[1]

[1] *NEI OGVFB*

[2] *NCATS*

[3] *NCI*

[4] *NEI*

Corresponding author's email: russell.quinn@nih.gov

We previously utilized 3D-Bioprinting to develop an in vitro model of the Outer Blood Retinal Barrier (OBRB). This model contains a fully-polarized retinal pigment epithelium (RPE) monolayer and an underlying dense capillary-bed resembling the choroidal vasculature. Using this OBRB model we replicated AMD-related pathological processes like choroidal neovascularization (CNV) and RPE-choroidal degeneration. The purpose of this study was to investigate the role of macrophages, a constituent cell type in the choroid, on vascular development and pathology by incorporating polarized human macrophages in this previously established model.

Endothelial cells, choroidal fibroblasts, and ocular pericytes were encapsulated in a collagen-derived gel and 3D-printed on a degradable scaffold to facilitate capillary formation. Induced pluripotent stem cell (iPSC)-RPEs were seeded on the apical side of the scaffold 7 days after bioprinting. Primary activated M1 (pro-inflammatory), M2(pro-angiogenic), and M1+M2 polarized macrophages were added to the tissue at the time of printing (day 0) or 28 days post printing.

Macrophages when added at day 0 as M1 or M2 single populations, or mixed populations of M1+M2, increased the speed of vascular maturation and also increased proangiogenic or inflammatory secretions. The greatest increase in maturation (described as length, width, and # of branch points) was achieved by combining macrophage subtypes, reflecting in-vivo behavior. Finally, macrophages were able to extend their lifespan to 3-4 weeks when embedded in tissues, as opposed to 7-10 days in 2D flasks. This 3D in vitro model may enable investigations into immune mechanisms influencing OBRB development in humans.

Abstract **#1718**

BIODEGRADABLE SMALL DIAMETER ATROMBOGENIC COATED VASCULAR GRAFTS SHOW HIGH PERMEABILITY IN SHEEP MODEL COMPARED TO EPTFE SYNTHETIC GRAFTS

Larisa V Antonova[P][1], Evgeniya O Krivkina[1], Andrey V Mironov[1], Maria A Rezvova[1], Sergey S Krutitskiy[1], Elena A Velikanova[1], Leonid S Barbarash[1]

[1] *Research Institute for Complex Issues of Cardiovascular Diseas, Kemerovo, Russian Federationes*

Corresponding author's email: antonova.la@mail.ru

Tissue-engineered vascular graft for the reconstruction of small arteries is still an unmet clinical need, albeit a number of promising prototypes have entered the preclinical development. Here we tested poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/poly(ε-caprolactone) 4 mm diameter vascular grafts equipped with vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and stromal cell-derived factor 1α (SDF-1α) and surface coated with heparin and iloprost (PHBV/PCL[VEGF-bFGF-SDF]Hep/Ilo, n = 8) in a sheep carotid artery interposition model, using biostable vascular prostheses of expanded poly(tetrafluoroethylene) (ePTFE, n = 5) as a control. Primary patency of PHBV/PCL[VEGF-bFGF-SDF]Hep/Ilo grafts was 62.5% (5/8) 24 hours postimplantation and 50% (4/8) 18 months postimplantation, while all (5/5) ePTFE conduits were occluded within the 24 hours after the surgery. At 18 months postimplantation, PHBV/PCL[VEGF-bFGF-SDF]Hep/Ilo grafts were completely resorbed and replaced by de novo vascular aneurysmatic tissue. Therefore, biodegradable PHBV/PCL[VEGF-bFGF-SDF]Hep/Ilo grafts showed better short- and long-term results than biostable ePTFE analogues, albeit these scaffolds must be reinforced for the efficient prevention of aneurysms.

Abstract **#1720**

THE RESPONSE OF HUMAN GINGIVAL FIBROBLASTS, KERATINOCYTES AND MACROPHAGES TO TI SURFACES PRE-INCUBATED WITH WHOLE BLOOD

William Arthur Lackington[1], Yvonne Elbs-Glatz[1], Stefanie Guimond[1], Katharina Maniura[1], Markus Rottmar[1]

[1] *Empa, Swiss Federal Laboratories for Materials Science and Technology*

Corresponding author's email: william.lackington@empa.ch

Classical in vitro testing platforms for implantable biomaterials offer limited predictability of clinical performance, which presents a major challenge in implant development. While classical platforms facilitate the study of biomaterial-tissue interfaces using relevant cell types, the importance of the first interaction a biomaterial encounters in vivo, that with whole human blood, is often neglected. In the context of titanium (Ti) implant soft tissue integration, for dental applications, the aim of this study was to assess how the blood-biomaterial interaction influences the tissue healing response. Clinically available Ti implant surfaces (machined, SLA, and SLActive) were used to study the response of relevant soft tissue cells (human gingival fibroblasts, gingival keratinocytes and macrophages), with and without a pre-incubation step in blood. Findings show that, depending on their surface properties, including rough-ness (Sa), contact angle and micro/nanostructures, distinct fibrin networks formed on the Ti surfaces after incubation with blood. Generally, across all cell types, pre-incubation of the Ti surfaces with blood led to enhanced attachment, metabolic activity, proliferation and matrix production. Ti surface properties were a key determinant of inflammatory marker (TNFα, IL-6 and IL-8) production by macrophages and pre-incubation of the Ti surfaces with blood led to significant changes in their relative amounts. Collectively, these findings indicate that while material surface properties play a large role in determining the tissue healing response, the interaction between a biomaterial and whole human blood can significantly alter this response. In conclusion, this study highlights that blood-biomaterial interaction should be taken into ac-count during implant development.

Abstract **#1721**

ANALYSIS OF THE STRUCTURAL AND FUNCTIONAL STATE OF THE LIVER TISSUE DURING LIVER REGENERATION BY THE FLIM AND TOF-SIMS METHODS

Svetlana Rodimovaᵽ[1,2], Daria Kuznetsova[1], Nikolai Bobrov[1,3], Alexander Gulin[4,5], Vadim Elagin[1], Maria Karabut[1], Vladislav Shcheslavskiy[1,6], Vladimir Zagainov[1,3], Elena Zagaynova[1,2]

[1] *Privolzhsky research medical university, Nizhny Novgorod, 603950 Russia*

[2] *N.I. Lobachevsky Nizhny Novgorod National Research State University, Nizhny Novgorod, 603950, Russia*

[3] *Volga District Medical Centre of Federal Medical and Biological Agency, Nizhny Novgorod, 603000, Russia*

[4] *N.N. Semenov Federal Research Center for Chemical Physics RAS, Moscow, 11999, Russia*

[5] *Lomonosov Moscow State University, Moscow, 119991, Russia*

[6] *Becker and Hickl GmbH, Berlin, 12277, Germany*

Corresponding author's email: srodimova123@gmail.com

The decrease of regenerative potential enhances the risk of postoperative liver failure. Standard clinical methods do not allow predicting the function of the remaining liver. Using modern label-free methods of multiphoton microscopy with the FLIM (fluorescence lifetime imaging) and SHG (second harmonic generation) it is possible to evaluate the metabolic state of hepatocytes during regeneration. In addition, time-of-flight secondary ion mass spectrometry (TOF-SIMS) provides information on the lipid and amino acid composition of the liver tissue.

The experiments were performed on Wistar rats weighing 400-500 g. Two models were selected- 70% parietal hepatectomy (PH) and 30% PH. Metabolic imaging was performed on 3th and 7th days after surgery. A separate analysis of NADH and NADPH was presented to evaluate the contribution of different metabolic pathways, as well as synthetic activity in hepatocytes. TOF-SIMS provide data on lipid composition of liver tissue.

As a result, a rise in the contribution of oxidative phosphorylation and biosynthetic processes in hepatocytes was shown, compensating energy consumption of hepatocytes during regeneration. Using TOF-SIMS it was shown an increase in the signal from fatty acids, sphingomyelin and cholesterol, in combination with a decrease in the signal from amino acid fragments.

The obtained parameters will be useful in determining the criteria for the intraoperative express assessment of the liver regenerative potential after surgery.

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Keywords

Liver regeneration; FLIM; TOF-SIMS

Abstract **#1723**

INVESTIGATING THE EFFECT OF CULTURE MEDIUM OSMOLARITY ON THE PHENOTYPE AND REGENERATIVE CAPACITY OF CANINE NUCLEUS PULPOSUS CELLS

Lisanne Laagland[P][1], Deepani Poramba-Lyanage[2], Frances Bach[2], Laura Creemers[3], Christine Le Maitre[4], Marianna Tryfonidou[2]

[1] *Utrecht University*

[2] *Utrecht University*

[3] *University Medical Centre Utrecht*

[4] *Sheffield Hallam University*

Corresponding author's email: l.t.laagland@uu.nl

Intervertebral disc (IVD) degeneration is associated with the loss of healthy matrix(1) and reduced osmolarity(2) within the nucleus pulposus (NP). Adjusting medium osmolarity represents a promising strategy for treating IVD degeneration since recent work demonstrated that increasing osmolarity during expansion exerted regenerative effects on human NP cells (NPCs) in vitro(3). Here, we investigated the effects of culture medium osmolarity on the identity and regenerative capacity of canine NPCs. Beagle NPCs were expanded in medium with standard osmolarity (300 mOsm/L) or adjusted to 400 or 500 mOsm/L. Following expansion, cells were re-differentiated for 14 days in 3D micro-aggregates (standard medium osmolarity). Osmolarity changes during cell expansion lead to changes in NPC morphology and growth rate, with cells expanded in 300 mOsm/L appearing more fibroblast-like, while at higher osmolarity the cells were sphere-shaped and at 500 mOsm/L showed a significantly decreased growth rate. At day 3 of the re-differentiation culture, micro-aggregates were harvested for gene expression analysis and NPCs expanded at 500 mOsm/L showed significantly increased expression of the osmolarity response gene NFAT5 and several NPC and progenitor markers (KRT18, CD73, CA12, FOXF1, ACAN) compared with NPCs expanded at 300 or 400 mOsm/L. Furthermore, expansion in 500 mOsm/L resulted in a significantly increased glycosaminoglycan and DNA content of the micro-aggregates compared to expansion at 400 mOsm/L, but not to 300 mOsm/L. In conclusion, expansion of NPCs in high osmolarity could have beneficial effects by selecting for a subpopulation of NPCs with a specific phenotype at the expense of the growth rate.

Keywords

Intervertebral disc degeneration; Osmolarity ; Cell-based treatments

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Abstract **#1724**

TARGETED PROTEOMIC ANALYSIS TO EXPLORE THE ANTI-INFLAMMATORY EFFECTS OF NOTOCHORDAL CELL-DERIVED MATRIX

Lisanne Laagland[P][1], Frances Bach[2], Frank Riemers[2], Laura Creemers[3], Markus Templin[4], Marianna Tryfonidou[2]

[1] *Utrecht University*

[2] *Utrecht University*

[3] *University Medical Centre Utrecht*

[4] *NMI TT Pharmaservices*

Corresponding author's email: l.t.laagland@uu.nl

Low back pain due to intervertebral disc (IVD) degeneration is a major health and socioeconomic problem throughout the world(1). In the young and healthy IVD, large and vacuolated notochordal cells (NCs) are present(2). These cells are, in some species (e.g. humans(2) and dogs(3)), replaced by chondrocyte-like nucleus pulposus cells (NPCs) during maturation and ageing. In previous studies porcine NC-derived matrix (NCM) induced regenerative and anti-inflammatory effects in human and bovine NPCs in vitro and canine IVDs in vitro and in vivo(4,5). However, since the precise mechanism behind NCM remains elusive, the aim of this study was to determine the mode of action of NCM. For this, canine NPCs were cultured with and without NCM. Afterwards, targeted proteomics was performed using DigiWest technology and results were confirmed in in vivo canine IVDs treated with NCM. DigiWest analysis showed that NCM mainly induced changes in the Mitogen-activated protein kinase (MAPK) pathway. The expression of key proteins downstream the MAPK pathway was mostly inhibited by NCM, such as p-ERK1/2, p-JNK/SAPK, and p-PKC. The expression of proteins that are known to dephosphorylate MAPK key signalling molecules, DUSP5 and DUSP6 was increased in NCM-treated canine NPCs. Confirming the DigiWest results, in vivo canine IVDs treated twice with NCM demonstrated increased DUSP5 protein expression compared with control IVDs. Taken together, these results indicate that NCM exerts its anti-inflammatory effects by increasing nuclear DUSP5 expression, which inactivates and dephosphorylates pERK1/2 and pPKC and by directly inactivating pPKC. This subsequently leads to reduced expression of inflammatory cytokines.

Keywords

Intervertebral disc degeneration; Notochordal cell-derived matrix; Anti-inflammatory effects

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Abstract **#1725**

THE HEALING OF WEIGHT-BEARING FEMORAL DEFECTS USING A LOW DOSE OF RHBMP-2 IS HIGHLY DEPENDENT ON THE BIOMATERIAL-BASED PLATFORM USED FOR LOCAL DELIVERY

William Arthur Lackington[P][1], Dominic Gehweiler[1], Ivan Zderic[1], Arlyng Gonzalez-Vazquez[2], Fergal O'Brien[2], Martin Stoddart[1], Keith Thompson[1]

[1] *AO Research Institute, Davos*

[2] *Royal College of Surgeons in Ireland*

Corresponding author's email: william.lackington@empa.ch

Traditional methods to promote bone healing (e.g. autograft) and advanced therapeutics (e.g. recombinant human bone morphogenetic protein-2 [rhBMP-2]) have been associated with performance limitations and dose-related safety concerns, respectively. Thus, there is an unmet need for osteoinductive bone graft substitutes that can support bone healing using lower doses of rhBMP-2. This study aimed to determine the therapeutic efficacy of low dose (5 µg) rhBMP-2 for inducing bone formation using: a thermoresponsive hyaluronic acid hydrogel doped with gelatin and laminin (HyA-GL), a collagen-only (CO), and a collagen-hydroxyapatite (CHA) scaffold. Efficacy over 14 weeks was evaluated using 2 mm segmental femoral defects in skeletally mature female F344 rats, internally fixated using a 1.25 mm-thick polyetheretherketone plate. Empty defects failed to bridge but administration of rhBMP- 2 using either CO or CHA scaffolds led to successful bridging, while using HyA-GL resulted in non-union. CHA scaffolds induced enhanced and stiffer new bone formation compared to both CO and HyA-GL scaffolds. Limited luminal remodeling occurred in both CO and CHA groups. Taken together, this study has determined that the healing of weight-bearing femoral defects using low dose rhBMP-2 is achievable, while its efficacy is highly dependent on the biomaterial-based platform used for local delivery.

Abstract **#1726**

ENGINEERING AN ON-CHIP VASCULAR-CARDIAC IN-VITRO MODEL TO STUDY CORONARY MICROVASCULAR DISEASE

Akinola D Akinbote$_P$[1], Kristina M Haase[1]

[1] *European Molecular Biology Laboratory Barcelona*

Corresponding author's email: akinola.akinbote@embl.es

Cardiovascular diseases (CVDs) are the single leading cause of deaths worldwide and are projected to be for the next 10 years. Owing to the high failure rates of bringing a drug to market, in part due to inadequate preclinical models, there is a pressing need to develop physiologically relevant platforms to study CVDs. Here, we present a novel vascularized cardiac tissue model to study coronary microvascular disease—responsible for about 42% of all CVDs. Utilizing only human cardiac primary cells, we developed functionally perfusable cardiac-specific vasculature. We evaluated endothelial barrier function, vessel morphology, stromal-endothelial cell interactions, as well as intra- and extra-vascular solute transport under different culture conditions. We found that while stromal cells were needed to develop non-leaky vessels, there is an optimal ratio of endothelial-to-stromal cells for developing functional (i.e perfusable) vessels. Moreover, we report dynamic vessel remodelling—defined by decreased vascular branches, increased vessel diameter, and quantified tight junction protein expression—in response to flow conditions. We present a 3D on-chip platform to study the coronary microvasculature in physiologically relevant in-vitro environments, a tool we are now optimizing for precision medicine using induced pluripotent stem cells.

Abstract **#1728**

EX VIVO MIGRATION OF CHONDROCYTES - CELL INVASION INTO ACELLULAR BIOMATERIALS FILLED IN A CARTILAGE RING MODEL IS DEPENDENT ON BIOMATERIAL COMPOSITION

Andrea Schwab[P][1], Marinus A. Wesdorp[2], Claudia Loebel[3], Riccardo Levato[4,5], Jason A. Burdick[3], Jos Malda[4,5], Gerjo J.V.M. van Osch[2], David Eglin[1], Matteo D'Este[1]

[1] *AO Research Institute Davos, Switzerland*

[2] *2.Erasmus MC, University Medical Center Rotterdam, the Netherlands*

[3] *3.Polymeric Biomaterials Laboratory, Department of Bioengineering, University of Pennsylvania, USA*

[4] *4.Biofabrication and Regenerative Medicine, Department of Orthopedics, University Medical Center Utrecht, the Netherlands*

[5] *5.Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands*

Corresponding author's email: andrea.schwab@aofoundation.org

Integrative cartilage repair requires the ability of chondrocytes to invade an acellular biomaterial implanted into the defect. Chondrocyte migration into the biomaterial is supported by good integration of the biomaterial to surrounding tissue in the defect.

The aim of this study was to compare cell migration of endogenous chondrocytes into semi-synthetic biomaterials with similar mechanical properties (1-5 kPa) using a cartilage explant model.

Full thickness cylindrical cartilage discs were isolated from bovine (6-month-old) patellae groove (diameter 8mm, thickness 1.5-2.5mm). Cartilage defects (diameter 4mm) were filled with cell-free hydrogels: collagen-I (col) or norbornene-functionalized hyaluronan (norHA) with dithiol (DTT, non-degradable) or MMP (degradable) crosslinker, tyramine-functionalized HA (THA), THA-col and gelatine-methacryloyl (GelMA 50% and 80% degree-of-functionalization DOF). Samples were cultured for 21 days in high-glucose DMEM containing 50ug/ml ascorbic acid and 10% FBS. Biomaterial integration, presence and location of chondrocytes were scored on Safranin-O-stained sections. A migration-index (MI) was developed and interobserver reliability, expressed as intraclass-correlation-coefficient (ICC, 1=excellent, 0=low reliability), was calculated.

Addition of col or lower DOF increased MI with highest MI-score for col (MI:6.9), followed by norHA-DTT, GelMA (50&80% DOF) and THA-col (MI: 3.0-5.3, ICC=0.926). THA and norHA-MMP showed least cell invasion (MI<1.7). Beside GelMA, biomaterials remained in the defect with majority of cells located at the biomaterial periphery. Chondrocytes migrated up to the defect center only in col (<0.5kPa) group.

Chondrocyte migration into biomaterials is a first step to improve cell-free approaches to repair cartilage defects with the aim for deposition of collagen II rich matrix and preventing fibrocartilage formation.

Abstract **#1729**

IMPACT OF IL-1B-PRIMED MSC ON WOUND HEALING: COMPARATIVE ANALYSIS OF TWO SOURCES

DEDIER Marianne$_P$[1,2], NIVET Muriel[1,2], MAGNE Brice[1,2], PELTZER Juliette[1,2], BANZET Sébastien[1,2], TROUILLAS Marina[1,2]

[1] *French Armed Forces Biomedical Research Institute, Clamart, France*

[2] *INSERM, U1197, Villejuif, France*

Corresponding author's email: marianne.dedier@inserm.fr

Despite several advances in skin tissue engineering and regenerative medicine, severe burns remain a major therapeutic issue. Mesenchymal Stromal Cells (MSC) have become a real therapeutic option in the field of tissue repair for their immunomodulatory and remodeling properties. This last years, these properties seem to be closely related to their secretory products such as proteins or small RNA. Recent discoveries have highlighted their sensitivity to external stimuli and therefore the possibility to orientate their secretory products to enhance their potential. Interleukin 1β (IL-1β) was used in a previous study as priming molecule, and IL-1β-primed gingival MSCs was shown to be superior to naive MSCs to improve wound healing and epidermal engraftment in vitro and in vivo.

The present study investigates whether bone marrow MSC respond similarly to IL-1β to favor wound healing through the secretion of proteins and miRNA. Naive and IL-1β-primed MSCs from gingival or bone marrow sources were evaluated in different in vitro models of migration, inflammation, dermal-epidermal junction formation. Preliminary results indicated that both MSC sources responds similarly to IL-1β priming and promote skin cell migration, favor extracellular matrix deposition and reduce inflammation in vitro. Their secretory profile for proteins and miRNA was evaluated using ELISA and RT-qPCR. We identified different important pro-healing actors induced by IL-1β priming as IL-6 and miR-146a-5p and tested their mechanisms of action in in vitro tests. These results underline the impact of IL-1β priming on MSC and its potential benefit in a burn wound treatment strategy.

Abstract **#1731**

HUMAN ADIPOSE TISSUE DERIVED MICROVASCULAR FRAGMENTS RETAIN VASCULAR CHARACTERISTICS IN 3D FIBRIN GEL CULTURE AFTER CRYOPRESERVATION

Egehan Salepci[1,2], Yourka D. Tchoukalova[1], Cathy S. Madsen[1], David G. Lott[1,3]

[1] *Head and Neck Regeneration Program, Mayo Clinic Center for Regenerative Medicine, Phoenix, AZ, USA*

[2] *Health Sciences University Sisli Hamidiye Etfal Research and Training Hospital, Istanbul. Turkey*

[3] *Department of Otorhinolaryngology-Head and Neck Surgery, Mayo Clinic, Phoenix, AZ, USA*

Corresponding author's email: egehansalepci@gmail.com

Aim and Background: Autologous adipose tissue derived microvascular fragments (ad-MVF) are shown to form functional vascular networks after implantation in animal models suggesting their potential to pre-vascularize tissue engineered constructs. Limited data is available regarding human ad-MVF1. We aimed to assess the behavior of human ad-MVF in vitro.
Methods: Human ad-MVF were isolated from abdominal lipoaspirate – as previously described in mice with minor modifications2. Ad-MVF were imaged with light microscopy, validated for viability (Hoechst and Propidium Iodide staining), and frozen. After 30 days, ad-MVF were thawed, resuspended in fibrinogen component of the fibrin sealant (TISSEEL), and transferred to 96-well plates (50 µl per well, containing 35,000 ad-MVF). Equal volume of thrombin was added to obtain gel consistency (Final concentration: 20 mg/ml fibrinogen and 2 IU/ml thrombin). At baseline and after 12 days of culture in endothelial medium, constructs were embedded in paraffin and stained for CD31 (endothelial), αSMA (pericyte) and ki67 (proliferation) markers.
Results: Ad-MVF were viable before and after cryopreservation. Sections at baseline contained ad-MVF that stained positive with both anti-CD31 and anti-αSMA. After 12 days of culture similar structures that still showed positivity for the both antigens were observed. They were lower in number but larger in size. Additionally, CD31 positive cells co-stained with ki67 suggesting cell proliferation and angiogenic activity.
Conclusion: Human ad-MVF survive cryopreservation and retain vascular characteristics after culture in fibrin gel suggesting they may be suitable to pre-vascularize tissue engineered constructs. Studies are in progress to test their angiogenic properties in vivo.

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Abstract **#1732**

THE INFLUENCE OF SCAFFOLD ARCHITECTURE AND COMPOSITION ON OSTEOBLAST-DERIVED EXTRACELLULAR VESICLES THERAPEUTIC EFFICACY FOR BONE REGENERATION

Kenny Man$_P$[1], Sophie Louth[1], Mathieu Brunet[1], David Hoey[2,3,4], Sophie Cox[1]

[1] *School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom.*

[2] *Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland.*

[3] *Dept. of Mechanical, Manufacturing, and Biomedical Engineering, School of Engineering, Trinity College Dublin, Ireland.*

[4] *Advanced Materials and Bioengineering Research Centre, Trinity College Dublin & RCSI, Ireland.*

Corresponding author's email: k.l.man@bham.ac.uk

Extracellular vesicles (EVs) are considered promising nanoscale therapeutics for bone regeneration. To date, EVs are procured from cells cultured on tissue culture plastic (TCP), limiting cell growth and not replicating conditions in situ. Studies have demonstrated the impact of 3D culture on promoting osteogenesis. Additive manufacturing has facilitated the fabrication of biomimetic environments. Therefore, this study investigated the influence of 3D printed scaffold architecture and composition on the therapeutic efficacy of osteoblast-derived EVs for bone repair.

Ti6Al4V titanium scaffolds with different pore sizes (500 and 1000 µm) and shapes (square and triangle) were fabricated by selective laser melting. Scaffolds were treated with/without a nano-needle hydroxyapatite (nnHA) coating. EVs were procured from scaffold-cultured osteoblasts over 2 weeks. EV size and concentration were defined using nanoparticle tracking analysis. Human bone marrow stromal cells (hBMSCs) osteogenic differentiation with scaffold-derived EVs was evaluated by qPCR, biochemistry and histological analysis.

Titanium scaffolds promoted osteoblast mineralisation compared to TCP. Scaffold-cultured osteoblasts secreted significantly enhanced EV quantity when compared to TCP, with scaffolds exhibiting larger pore sizes (1000 µm) and permeabilities (triangle) promoting EV yield. Moreover, osteoblast-derived EVs isolated from triangle pore scaffolds significantly increased hBMSCs mineralisation when compared to EVs from square pore scaffolds and TCP. Interestingly, nnHA coating significantly improved osteoblast mineralisation, EV production and EV-induced hBMSCs mineralisation compared to uncoated scaffolds.

Together, these findings demonstrate the impact of scaffold architecture and composition on EV therapeutic efficacy, indicating the potential of bone-mimetic culture platforms to enhance the production of pro-regenerative EVs for bone repair.

Abstract **#1733**

A NOVEL MODEL TO STUDY OSTEOCHONDRAL TISSUE DAMAGE RESPONSES IN PTOA

Isabel Amado$_P$[1,2]

[1] *Royal College of Surgeons in Ireland*

[2] *Trinity College Dublin*

Corresponding author's email: isabelamado@rcsi.com

Post-traumatic osteoarthritis (OA) is a disease subset, which leads to joint changes [1] after acute mechanical joint injury. While moderate mechanical loads are essential for maintaining tissue health; excessive mechanical damage can result in pathological changes in both cartilage [2] and subchondral bone (such as microdamage) [3]. How the initial damage, and subsequent responses, occur and whether they are inter-dependent or not is an important, but challenging, question. Here we propose a novel patellar explant to study osteochondral tissue dynamics. Existing models (femoral head, osteochondral plugs) require direct tissue damage during harvest – this presents a challenge when initial tissue damage is the object of study. Thus, we exposed patellar explants both to chemical and mechanical damage, and then assessed tissue damage responses using a series of assays. For chemical damage, explants were treated with IL-1B for 0, 1, 3 and 7 days, while mechanical damage was generated by compression to 10 % strain for 10 cycles at 0.5Hz, at the same time points. In both cases, Sulfated glycosaminoglycan (sGAG), and matrix metalloproteases 13 (MMP-13) were measured to assess cartilage responses whilst alkaline phosphatase (ALP) assessed bone responses. Our data show that patellar explants are viable for at least 28 days. Explants treated with IL-1B showed that both proteoglycan content and bone metabolism markers were increased ($p<0.05$) after 7 days. Furthermore, mechanical damage caused a significant increase in MMP-13. In summary, we demonstrate a novel patellar explant model that can be a useful tool to study bone-cartilage crosstalk in PTOA.

Keywords

Bone; Cartilage ; post-traumatic osteoarthritis

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Abstract **#1734**

THIN MELT-ELECTROWRITTEN SCAFFOLDS AS A NOVEL ALTERNATIVE FOR POROUS INSERTS IN BLOOD-BRAIN BARRIER MODELS IN VITRO

Magdalena Gladysz[1,2], Marleen Kamperman[1], Anika Nagelkerke[2], Malgorzata Wlodarczyk-Biegun[1]

[1] *Faculty of Science and Engineering, Polymer Science — Zernike Institute for Advanced Materials University of Groningen Nijenborgh 4, 9747 AG Groningen The Netherlands*

[2] *Faculty of Science and Engineering, Pharmaceutical Analysis — Groningen Research Institute of Pharmacy University of Groningen Antonius Deusinglaan 1 9713 AV Groningen The Netherlands*

Corresponding author's email: m.z.gladysz@rug.nl

The blood-brain barrier (BBB) is a semipermeable part of the central nervous system's microvasculature, separating the blood from brain tissue. It consists of an endothelial cell layer covered with pericytes and astrocytes, which together protect the brain from non-selective substances, including pathogens but also therapeutics, e.g. against cancer. Therefore, recreating this environment in vitro in order to characterize and enhance drug uptake and distribution is imperative.
Here, melt electrowriting (MEW) is used to fabricate a thin scaffold on which endothelial cells are encouraged to form a confluent, monolayer. MEW has been chosen because of its unprecedented accuracy and precision. Scaffold design and properties, such as fibre thickness, pore size, number of layers, can be adjusted and modified up to the micrometric range and tailored to support an adequate cellular response. The aim is to obtain a tight monolayer, favouring cell-cell interactions, which is a feature that conventional inserts, commonly used for BBB models, lack.
Scaffolds were manufactured from medical grade poly(ε-caprolactone). They were seeded with relevant cell types and their designs were tailored to support the cellular response, favouring the desired growth morphology. Cell behaviour along with tissue formation was investigated via live and fluorescent imaging techniques. Membrane permeability was also assessed.
The future steps will include co-cultures and the design of a customised microfluidic device for drug screening studies. Further development of this BBB model will improve the accuracy of the final in vitro microfluidic system which could be used in preclinical studies to improve drug uptake.

Abstract **#1735**

NANOSTRUCTURED DENSE COLLAGEN-POLYESTER COMPOSITE HYDROGELS AS AMPHIPHILIC PLATFORMS FOR DRUG DELIVERY

Christophe Hélary[P][1], Xiaolin Wang[1,2], Olivier Ronsin[3,4], Basile Gravez[5], Nicolette Farman[5], Tristan Baumberger[3,4], Frédéric Jaisser[5], Thibaud Coradin[1]

[1] Sorbonne Université, CNRS, UMR 7574 - Laboratoire de Chimie de la Matière Condensée de Paris - F-75005 Paris, France

[2] School of Pharmacy and State Key - Laboratory of Quality Research in Chinese Medicine - Macau University of Science and Technology, Taipa, Macao, China

[3] Sorbonne Université, CNRS - Institut des NanoSciences de Paris, INSP - F-75005 Paris, France

[4] Université de Paris

[5] INSERM - Centre de Recherche des Cordeliers - Sorbonne Université -Université de Paris - F-75005 Paris, France.

Corresponding author's email: christophe.helary@upmc.fr

Collagen based hydrogels are broadly used in tissue engineering as they are biocompatible, biodegradable and allow adhesion of conjonctive cells. Unfortunately, these biomaterials are poor drug delivery systems, thereby limiting their utilization to prevent infection or modulate inflammation1. Associating collagen with biodegradable hydrophobic polyesters constitutes a promising method for the design of medicated biomaterials. Current collagen-polyester composite hydrogels consisting of pre-formed polymeric particles encapsulated within a low concentrated collagen hydrogel suffer from poor physical properties and low drug loading. Herein, an amphiphilic composite platform associating dense collagen hydrogels (40 mg/mL) and up to 50 wt% polyesters with different hydrophobicity and chain length was developed. An original method of fabrication was disclosed based on in situ nanoprecipitation of polyesters impregnated in a pre-formed 3D dense collagen network. Composites made of PLGA and PLA but not PCL exhibited improved mechanical properties compared to those of pure collagen dense hydrogels while keeping a high degree of hydration. Release kinetics of Spironolactone, a lipophilic steroid used as a drug model, could be tuned over one month. No cytotoxicity of the composites was observed on fibroblasts and keratinocytes and spironolactone remained active after release from composites. Unlike the incorporation of pre-formed particles, the new process allows for both improved physical properties of collagen hydrogels and controlled drug delivery. The ease of fabrication, wide range of accessible compositions and positive preliminary safety evaluations of these collagen-polyesters will favour their translation into clinics in wide areas such as drug delivery and tissue engineering.

Keywords

Dense collagen hydrogels; Composites; In situ nanoprecipitation

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Abstract **#1736**

THE IPSPINE PROJECT: LEARNING TO INTEGRATE ETHICS IN A MULTIDISCIPLINARY SCIENTIFIC SETTING

Marianna Tryfonidou[P][1]

[1] *Faculty of Veterinary Medicine, Utrecht University, the Netherlands*

Corresponding author's email: M.A.Tryfonidou@uu.nl

In the iPSpine Horizon 2020 project 21 partners joined together to develop an ATMP for the treatment of chronic low back pain caused by disc degeneration. Key opinion leaders joined forces with academic partners, university medical centers, research institutes, and end users represented by a patient representative foundation and companies. Induced pluripotent stem (iPS) cells are differentiated into notochordal cells, specialized cells that only reside in juvenile discs. They are combined with smart biomaterials to provide them with the necessary instructions and support to thrive within the degenerate disc. Hereby iPSpine hopes to achieve biologic disc repair and ultimately offer improved quality of life for millions of patients, through long-lasting reduction of low back pain.

These technological advances will be supplemented with recommendations on ethical and regulatory affairs to enhance further development and implementation of iPSC-based therapies. In that respect, the consortium contributed into mapping of hard and soft impacts to widen the understanding of the ethical implications of stem cell research at different levels (scientists, regulators, patients). Furthermore, the partners receive training and advice by regulatory experts to meet the demands of the regulatory field of ATMPs during development up to proof-of-concept in clinically relevant animal models. During the keynote I will elude on my experiences as coordinator of such a multidisciplinary project and how we managed to integrate ethics and humanities in its scientific context with the ultimate aim to set an example on long term, fast forwarding, preclinical research that is closer to the patient in need.

Abstract **#1737**

DRUG ELUTING 3D PRINTED SCAFFOLDS FOR BONE TISSUE ENGINEERING

Jaidev Chakka$_P$[1], David Nakhla[1], Sanjeeb Saha[1], Satheesh Elangovan[1], Aliasger Salem[1]

[1] *University of Iowa*

Corresponding author's email: jaidev-chakka@uiowa.edu

Bone regeneration is a complex mechanism, and the application of growth factors will accelerate the regeneration process. Ferulic acid (FA) is a small molecule of Chinese herbal medicine that has bone regeneration properties. The sustained release of these growth factors is critical for the long-term new bone formation at the defect site. In the present study, the FA was mixed with polylactide-co-glycolide (PLGA) and extruded using a twin-screw hot-melt extruder. The extruder polymer-drug composition was fed into the thermoplastic canister that fits into the bioprinter's print head (Cellink, Sweden) and printed into 8x2 (diameter x height) mm porous structures. The PLGA-FA scaffolds delivered a sustained release of FA for seven days. The scaffolds were implanted into 8 mm critical calvarial defects of Fisher344 male rats. The rats were euthanized after 30 days, and the bone regeneration was analyzed using microCT and H&E histology for bone volume and new bone quantification. The results showed that the PLGA-FA scaffolds showed significant BV/TV compared to PLGA and empty-defect groups.

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Abstract **#1738**

TISSUE AND ORGAN SCAFFOLD PRODUCTION BY SUPERCRITICAL CARBON DIOXIDE EXTRACTION TECHNOLOGY FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Dar-Jen Hsieh[P][1], Srinivasan Periasamy[1], Ko-Chung Yen[1], Yi-Chun Yeh[1], Yun-Ju Chen[1], Hung-Chou Wang[1], Yih-Wen Tarng[2]

[1] *R&D Center, ACRO Biomedical Co., Ltd. Luzhu District, Kaohsiung City 82151, Taiwan*

[2] *Department of Orthopedics, Kaohsiung Veterans General Hospital, Kaohsiung City, Taiwan.*

Corresponding author's email: dj@acrobiomedical.com

Tissue engineering and regenerative medicine (TERM) is an emerging field focusing on the development of alternative therapies for tissue and organ repair by merging tissue and organ bioactive scaffolds, cells, and signal molecules into functional tissues. Tissue and organ scaffolds play a vital role and act as a crucial player in the game of 'TERM'. In the current study, we employed supercritical carbon dioxide (SCCO2) technology to produce tissue and organ scaffolds for experimental and clinical tissue engineering and regenerative medicine. We used proprietary SCCO2 extraction technology to produce dermis, whole skin, cancellous bone, heart, liver, brain, kidney, pancreas, blood vessel, ureter from a porcine source. The tissues and organs were washed in saline followed by subjecting the tissues to SCCO2 at 100-350 bar carbon dioxide pressure, 20-40°C and 4-12 h finally washed with sodium hydroxide and water. Decellularized tissue and organ scaffolds were stained by hematoxylin and eosin and 4,6-diamidino-2-phenylindole (DAPI) staining and scanning electron microscopy (SEM) depicted complete removal of cells. SCCO2 produced organ scaffold is a bioartificial scaffold that maintains the structure of the extracellular matrix. Our tissue and organ scaffolds are biologically mimetic scaffolds such as biologically active ECM thus creates a in vivo-like microenvironment mimicking biological entities and stimulating cell-specific responses to lead to tissue regeneration and repair. To conclude, we developed and efficient process to produce tissue and organ scaffolds from porcine origin. These organ scaffolds can be used in experimental and clinical tissue engineering and regenerative medicine.

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Abstract **#1739**

A NOVEL HISTOTYPIC 3D CONSTRUCT FOR RHINOPLASTY PRODUCED USING SUPERCRITICAL CARBON DIOXIDE DECELLULARIZED PORCINE NASAL CARTILAGE GRAFT CULTURED WITH CHONDROCYTE.

Su-Shin Lee[P][1], Yi-Chia Wu[1], Shu-Hung Huang[1], Ying-Che Chen[2], Srinivasan Periasamy[3], Dar-Jen Hsieh[3], Yi-Chun Yeh[3], Yi-Ping Lai[3], Yun-Nan Lin[1]

[1] *Division of Plastic Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan*

[2] *Department of Surgery, Kaohsiung Municipal Siaogang Hospital, Kaohsiung, Taiwan*

[3] *R&D Center, ACRO Biomedical Co., Ltd. Luzhu District, Kaohsiung City 82151, Taiwan*

Corresponding author's email: dj@acrobiomedical.com

Rhinoplasty is the most difficult aesthetic surgical procedures with a high rate of revision. Rhinoplasty adjusts and reorganizes the defected nose caused by trauma, therapeutic operations including cancer. However, we developed a bioactive scaffold with 3D histotypic cartilage culture using supercritical (SCCO2) carbon dioxide decellularized porcine nasal cartilage graft (dPNCG). dPNCG was produced by proprietary SCCO2 (100-350 bar carbon dioxide pressure, 20-40°C) extraction technology from porcine nasal cartilage. An experimental 3D histotypic culture was engineered using dPNCG, rat adipose-derived stem cells (ADSC) and chondrocytes with different percentage of cells and cultured for 21 days. dPNCG complete decellularization was characterized by hematoxylin and eosin (H&E), DAPI, alcian blue staining, scanning electron microscopy and residual DNA content. A solid mass of 3D histotypic cartilage with significant production of glycosaminoglycans in dPNCG with 100% chondrocytes culture. H&E and alcian blue staining showed an intact tissue mass, with cartilage granules bound to one another by extracellular matrix and proteoglycan, to form a 3D structure. Besides, phenotype chondrogenic markers, type II collagen, aggrecan and SOX-9 were elevated indicating chondrocytes cultured on dPNCG substrate synthesized type II collagen along with extracellular matrix to produce 3D histotypic cartilage. To conclude, dPNCG is an excellent substrate bioactive scaffold that might offer a suitable environment for human nasal chondrocytes to produce 3D histotypic cartilage and a promising potential candidate for cartilage tissue engineering in rhinoplasty.

Abstract **#1740**

DIRECT IN VIVO RECELLULARIZATION OF SUPERCRITICAL CARBON DIOXIDE DECELLULARIZED ARTERY.

Shih-Ying Sung$_P$[1], Chien-Sung Tsai[1], Yi-Ting Tsai[1], Yi-Wen Lin[2], Feng-Yen Lin[3], Ko-Chung Yen[4], Srinivasan Periasamy[4], Dar-Jen Hsieh[4]

[1] Division of Cardiovascular Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

[2] Institute of Oral Biology, National Yang-Ming University, Taipei, Taiwan

[3] 3Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

[4] R&D Center, ACRO Biomedical Co., Ltd. Luzhu District, Kaohsiung City 82151, Taiwan

Corresponding author's email: dj@acrobiomedical.com

Tissue-engineered vascular graft commonly comprised of a scaffold, seeded cells, and the appropriate signals to encourage vascular tissue formation. However, we proposed the direct approach to use supercritical carbon dioxide (SCCO2) technology decellularized rabbit blood artery and implant directly into the rat artery to evaluate the main concept of tissue engineering, the decellularization-recellularization approach. Rabbit femoral artery was harvested from a healthy New Zealand white rabbit. SCCO2 extraction technology was used to decellularize the rabbit artery. By subjecting the artery to SCCO2 at 100-350 bar carbon dioxide pressure, 30-40°C and 0.5-2 h finally washed with sodium hydroxide and water completely removed the cells. The decellularized artery was implanted in August Copenhagen Irish (ACI) rats. Doppler ultrasonography depicted implanted decellularized blood vessel showed normal blood flow in all examinations after 12, 42 and 58 days. Recellularization was complete as depicted by hematoxylin and eosin staining. Endothelial cell distribution in the recellularized blood vessel was evaluated by the endothelial marker. Mild expression of inflammatory markers was noticed, indicating regeneration. Cell distribution was noticed in all three layers of the blood vessel, with the expression of alpha-smooth muscle actin. Endothelial markers such as CD34 and vWF were expressed on the implanted artery. To conclude, SCCO2 decellularized rabbit artery direct implantation to rats depicted excellent patency and excellent recellularization with the normal function of the artery.

Abstract **#1741**

ROLE OF SUPERCRITICAL CARBON DIOXIDE DECELLULARIZED PORCINE CARTILAGE GRAFT IN THE REGENERATION OF ARTICULAR CARTILAGE IN ACLT-INDUCED OA RATS.

Chia-Chun Wu[P][1], Yih-Wen Tarng[2], Dur-Zong Hsu[3], Srinivasan Periasamy[4], Yi-Chun Yeh[4], Yi-Ping Lai[4], Dar-Jen Hsieh[4]

[1] *Department of Orthopedics, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*

[2] *Department of Orthopedic, Kaohsiung Veterans General Hospital, Kaohsiung city, Taiwan.*

[3] *Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

[4] *R&D Center, ACRO Biomedical Co., Ltd. Luzhu District, Kaohsiung City 82151, Taiwan*

Corresponding author's email: dj@acrobiomedical.com

Osteoarthritis (OA) is the most common articular cartilage degenerative joint disease and a major cause of pain and functional disability in humans. Cartilage tissue engineering (CTE) is to use supercritical carbon dioxide (SCCO2) decellularized porcine cartilage graft as a biomaterial scaffold, plasma rich platelet (PRP) as bioactive molecules, and chondrocytes from rat as the cells to cure the injury and regenerate new cartilage in anterior cruciate ligament transection (ACLT)-induced OA model. We used proprietary SCCO2 (100-350 bar carbon dioxide pressure, 20-40°C) extraction technology to produce decellularized porcine cartilage graft from porcine articular cartilage. SCCO2 decellularized porcine cartilage graft (dPCG) characterized by hematoxylin and eosin and 4,6-diamidino-2-phenylindole (DAPI) staining and scanning electron microscopy (SEM) depicted complete removal of cells. Intra-articular administration of dPCG with or without PRP is efficient in attenuating the progression and repairing the damaged cartilage. In addition, dPCG with and/or without PRP significantly reduced the ACLT-induced OA symptoms and attenuated the OA progression. dPCG reduced pain improved articular cartilage damage in the rat knee characterized by X-ray and micro-CT. Besides, dPCG significantly increased and modulated type II collagen, aggrecan and SOX-9 expression and attenuated the articular knee cartilage damage. To conclude, dPCG attenuated ACLT-induced OA progression with and/or without PRP by elevating the expression of type II collagen, aggrecan and SOX-9. The present investigation might serve as a potential therapy for OA patients and patients with cartilage defect caused by the sport-related injury.

Abstract **#1743**

SUPERCRITICAL CARBON DIOXIDE DECELLULARIZED PORCINE CARTILAGE ENGINEERED 3D COMPOSITE FOR ARTICULAR CARTILAGE REGENERATION.

Yi-Ting Chen[P][1], Herng-Sheng Lee[2], Dar-Jen Hsieh[3], Srinivasan Periasamy[3], Yi-Chun Yeh[3], Yi-Ping Lai[3], Yih-Wen Tarng[4]

[1] *Department of Orthopedic, Kaohsiung Veterans General Hospital, Kaohsiung City, Taiwan*

[2] *Department of Pathology, Kaohsiung Veterans General Hospital, Kaohsiung City, Taiwan*

[3] *R&D Center, ACRO Biomedical Co., Ltd. Luzhu District, Kaohsiung City 82151, Taiwan*

[4] *Department of Orthopedics, Kaohsiung Veterans General Hospital, Kaohsiung City, Taiwan.*

Corresponding author's email: dj@acrobiomedical.com

Articular cartilage has limited repair, regenerative capacity and a challenging clinical issue. Thus there is a continued search for the procedures and devices, to explore cell sources and tissue engineering methods for cell-based therapies, to restore articular cartilage. This study aimed to construct a novel 3D composite with decellularized cartilage for the treatment of cartilage defects.

Supercritical carbon dioxide (scCO2) was used to decellularize porcine articular cartilage. The 3D composite was engineered using 50, 100, or 250 mg scCO2 decellularized cartilage scaffolds with PRP, thrombin, and porcine chondrocytes. Expression of SOX-9, collagen type II, and aggrecan was evaluated in the 3D composite employing immunohistochemistry. In addition, 3D composites were made using with and without agarose along with decellularized cartilage scaffolds to elucidate the role of decellularized cartilage matrix on chondrocyte proliferation. Chondrocyte proliferation was optimal in 100mg growth decellularized cartilage, relative to 50 and 250mg. The chondrocyte growth and expression of collagen type II, SOX-9, and aggrecan were significantly elevated in the 3D composite with decellularized cartilage than without decellularized cartilage. The 3D composites with decellularized cartilage with agarose gel, showed exceptional chondrocytes growth, compared to agarose gel without decellularized cartilage. To conclude 3D composite made of decellularized cartilage scaffold acts as a scaffold substrate for the chondrocyte with native type II collagen, thus facilitating ECM production by chondrocytes. This new cell-scaffold construct may provide the basis of a viable chondral graft suitable for in vivo implantation.

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Abstract **#1744**

PROMOTION OF OSSEOINTEGRATION OF TITANIUM IMPLANTS IN OSTEOPOROTIC RATS BASED ON TITANIUM AFFINITY BIOMIMETIC POLYPEPTIDE

Qin Shi[P][1], Huan Zhao[1], Guoqing Pan[2]

[1] *Department of Orthopaedics, The First Affiliated Hospital of Soochow University*

[2] *School of Materials Science and Engineering, Jiangsu University*

Corresponding author's email: shiqin@suda.edu.cn

Introduction: In the previous study, we aim to study whether titanium affinity biomimetic peptide DOPA-BMP-2 could promote the osteogenesis of bone marrow mesenchymal stem cells (BMSCs) in vitro and accelerates the osseointegration of titanium screws in vivo in osteoporotic rats.

Methods: Titanium sheets and titanium screws were dual-functionalized by soaking to graft DOPA-BMP-2 and DOPA-RGD bioactive polypeptide. The physicochemical properties was explored and the optimal concentration of DOPA-BMP-2 polypeptide and DOPA-RGD polypeptide (promoting cell adhesion) were studied to promote the osteogenesis of BMSCs in vitro. Then, titanium screw coated with dual peptides were implanted into the osteoporotic rats to comprehensively evaluate the osseointegration.

Results: The results of X-ray photoelectron spectroscopy, water contact angle and atomic force microscopy displayed that the DOPA-BMP-2 and DOPA-RGD polypeptides were successfully grafted onto the titanium surface. QRT-PCR, ALP staining and alizarin red staining results further confirmed that the ratio of BMP-2 and RGD at 3:1 (BMP-2:RGD=3:1) had the strongest bone-promoting of BMSCs in vitro. In bone-implant contact (BIC) area of the osteoporotic rats, quantitative parameters of Micro CT showed that the bone volume/tissue volume, trabecular bone number, trabecular bone thickness of dual-functionalized group were higher than other groups, trabecular bone separation and trabecular pattern factor were decreased. The toluidine blue staining and the anchorage force test showed that the dual-functionalized titanium affinity biomimetic peptide promoted osseointegration in vivo.

Conclusions: Biomimetic active peptides and dual-functionalized strategies have successfully promoted osseointegration of titanium implants in osteoporotic rats.

Abstract **#1745**

PEPTIDE TARGETED CORE CROSS-LINKED MICELLES FOR DOX DELIVERY TO HER2 EXPRESSING CANCER CELLS

Nazende Nur Bayram$_P$[1], Gizem Tuğçe Ulu[2], Seda Gürdap[1], İsmail Alper İşoğlu[1], Yusuf Baran[2], Sevil Dinçer İşoğlu[1]

[1] *Abdullah Gul University, Bioengineering Department, Kayseri, Turkey*

[2] *İzmir Instıtute of Technology, Molecular Biology and Genetic Department, İzmir, Turkey*

Corresponding author's email: nazendeaksit@gmail.com

In this study, we prepared a novel targeted and extra stable micellar nanocarrier that can facilitate intracellular drug release. First, ((N-3-sulfopropyl-N, N-dimethylammonium)ethyl methacrylate was synthesized by RAFT polymerization, and it was followed by copolymerization of macroCTA with AEM in the presence of an acid-degradable cross-linker. Then, a peptide estimated by phage display for HER-2 recognition was incorporated into these core cross-linked micelles with carbodiimide reaction. Following this, DOX was loaded to the micelle nanocarriers by dialysis method in varying amounts to reach optimal loading. The resulted formulations were characterized in terms of size and zeta potential values. For polymer characterization, 1H-NMR, 13C-NMR, FTIR, and GPC were used. Size and morphological characterizations of the micelle nanocarriers were performed by Zeta Sizer and TEM, respectively. Analyses demonstrated that micelles with very low size distribution with sizes below 100 nm were obtained successfully. Then, pH-triggered DOX release was achieved due to the acid-degradable cross-links. Afterward, the efficiency of drug-loaded and targeted micelles on SKBR-3 cell lines was determined by using cell viability assay with the healthy breast cells as control (MCF-10A). Drug loaded micelles interacted with SKBR3 and MCF-10A cell lines for toxicity and drug efficiency, and the results were evaluated. According to the in vitro studies, the cytotoxicity of DOX loaded, peptide conjugated micelles were higher than free DOX at the same drug concentration, which indicates the high efficiency and selectivity of a peptide targeted smart nanocarrier system to HER2 positive breast cancer cells. Acknowledgment: This work is supported by the Scientific and Technological Research Council of Turkey(TÜBİTAK), Project Number: 216S639

Keywords

core cross-linked micelles; breast cancer; HER2 targeting

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Abstract **#1746**

EGGSHELL MEMBRANE AS A BIOACTIVE AGENT IN POLYMERIC NANOTOPOGRAPHIC SCAFFOLDS FOR ENHANCED BONE REGENERATION

Yonghyun Gwon[P][1]

[1] *Chonnam National University*

Corresponding author's email: gyhjhj0716@gmail.com

A bone regeneration scaffold is typically designed as a platform to effectively heal a bone defect while preventing soft tissue infiltration. Despite the wide variety of scaffold materials currently available, such as collagen, critical problems in achieving bone regeneration remain, including a rapid absorption period and low tensile strength as well as high costs. Inspired by extracellular matrix protein and topographical cues, we developed a polycaprolactone-based scaffold for bone regeneration using a soluble eggshell membrane protein (SEP) coating and a nanotopography structure for enhancing the physical properties and bioactivity. The scaffold exhibited adequate flexibility and mechanical strength as a biomedical platform for bone regeneration. The highly aligned nanostructures and SEP coating were found to regulate and enhance cell morphology, adhesion, proliferation, and differentiation in vitro. In a calvaria bone defect mouse model, the scaffolds coated with SEP applied to the defect site promoted bone regeneration along the direction of the nanotopography in vivo. These findings demonstrate that bone-inspired nanostructures and SEP coatings have high potential to be applicable in the design and manipulation of scaffolds for bone regeneration.

Abstract **#1747**

NANOSCALE TOPOGRAPHIES TO ENHANCE MESENCHYMAL STEM CELL ADHESION AND REDUCE BIOFILM FORMATION

Matthew Dalby[P][1]

[1] *University of Glasgow*

Corresponding author's email: matthew.dalby@glasgow.ac.uk

Post-operative infection is a major complication in patients recovering from orthopaedic surgery. As such, there is a clinical need to develop biomaterials for use in regenerative surgery that can promote mesenchymal stem cell (MSC) osteospecific differentiation and that can prevent infection caused by biofilm-forming pathogens. Nanotopographical approaches to pathogen control are being identified, including in orthopaedic materials such as titanium and its alloys. These topographies use high aspect ratio nanospikes or nanowires to prevent bacterial adhesion but these features puncture adhering cells, thus also reducing MSC adhesion. Here, we we will describe a number of approaches that can enhance MSC interaction without losing reduction in bacterial adhesion. An example is using poly(ethyl acrylate) (PEA) polymer coatings on titanium nanowires to spontaneously organise fibronectin (FN) and to deliver bone morphogenetic protein 2 (BMP2) to enhance MSC adhesion and osteospecific signalling. This nanotopography when combined with the PEA coating enhanced osteogenesis and reduced adhesion of Pseudomonas aeruginosa in culture. Using a novel MSC–Pseudomonas aeruginosa co-culture, it can also be shown that the coated nanotopographies protect MSCs from cytotoxic quorum sensing and signalling molecules. Such approaches may be useful in development of improved orthopaedic fixation.

Abstract **#1748**

FABRICATION OF VASCULAR NETWORK MIMICKING PATTERN USING FLEXOGRAPHIC PRINTING TECHNOLOGY.

Anna Fritschen[P][1], Pauline Brumm[1], Lara Doss[1], Edgar Dörsam[1], Andread Blaeser[1,2]

[1] *Institute for Printing Science and Technology, Technical University of Darmstadt, Germany*

[2] *Centre for Synthetic Biology, Technical University of Darmstadt, Germany*

Corresponding author's email: fritschen@idd.tu-darmstadt.de

In tissue engineering, the inclusion of vascular networks or other nutritional channels has become of major importance, as necrosis would otherwise limit the thickness of engineered tissue to hundreds of micrometers.
In order to fabricate such networks, multiple approaches exist, including soft-lithography, casting approaches and 3D-printing. However, hitherto applied approaches are either limited in production speed, require intensive manual work or entail a limited resolution. Additionally, fabrication of truly biomimetic vascular networks, whose scale span multiple orders of magnitude (micro- to millimeters), is extremely challenging.
To overcome the described limitations, we employed flexographic printing technology for fast and high-resolution fabrication of vascular network mimicking patterns made of gelatin. Dendritic and highly biomimetic hydrogel structures that mimic the vascular network in a human body can be fabricated with high speed (up to 1.5 m/s), on large areas (50 x 100 mm2) and covering a broad vascular size spectrum (30 µm–0.6 mm). Height, width, degree of branching and connectivity of the printed network were tailored by the gelatin concentration (5–30 %), print substrate selection and printing parameters (pressure, anilox shape, temperature).
Following printing the gelatin structures were embedded in various types of hydrogels, including agarose and agarose-gelatin blends, as sacrificial master molds. Bonding of the casted gels onto a substrate led to bioinspired, perfusable networks. In future, these can be applied as nutrient and oxygen providing channels.
In summary, our work is a major step towards rapid, cost-efficient and large-scale fabrication of vascularized tissues, applicable to a variety of hydrogels.

Keywords

flexographic printing; biomimetic networks; vascularization

Abstract **#1749**

DESIGNING BIOINK AND BIOASSEMBLY PLATFORMS FOR MUSCULOSKELETAL REGENERATIVE MEDICINE

Tim Woodfield[P][1]

[1] *University of Otago Christchurch*

Corresponding author's email: tim.woodfield@otago.ac.nz

Biofabrication technologies, including 3D bioprinting and bioassembly, enable the generation of engineered constructs that replicate the complex 3D organization of native tissues via automated hierarchical placement of cell-laden bioinks, tissue modules, and/or bioactive factors. Photo-initiated radical polymerization combining light and photo-initiators to generate radicals for crosslinking photo-polymerizable macromers, has been widely employed in 3D bioprinting of cell-laden hydrogels. Despite rapid advances in biofabrication technologies, no universal bioink exists. The major challenge for translational regenerative medicine is that the processing requirements (biofabrication window) of current bioinks is narrow, requiring optimization of each bioink for each individual biofabrication technique and specific tissue niche e.g. high viscosity "shear thinning" bioinks necessary for extrusion bioprinting versus low viscosity bioinks for lithography-based bioprinting.

This presentation discusses alternative strategies to provide highly tunable bioinks that 1) promote a specific cell-instructive niche using light activated crosslinking in high throughput modular spheroids, and 2) are printable across multiple biofabrication technologies, including extrusion-, lithography- and microfluidic-based bioprinting.

This talk will describe the design of versatile, photo-clickable bioinks and bioresins for biofabrication of 3D in vitro models targeting cartilage, bone and vascular network regeneration. We describe our experiences in developing hybrid tissue constructs and convergence with 3D spheroid bioassembly platforms for controlling multicellular spheroid fusion, extracellular matrix (ECM) formation and stem cell niche, offering new paradigms for high-throughput screening, "on-chip" and osteochondral tissue repair applications.

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Abstract **#1750**

MELT ELECTROWRITTEN SCAFFOLDS FOR SOFT-TO-HARD TISSUE INTERFACES: MECHANICAL AND BIOLOGICAL EVALUATION

Piotr Zielinski[1], Marleen Kamperman[1], Malgorzata Wlodarczyk-Biegun[1]

[1] *Polymer Science - Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands*

Corresponding author's email: p.s.zielinski@rug.nl

Musculoskeletal injuries are one of the main causes of disability worldwide. Soft and hard tissues are integrated through gradient interfaces to form the complex system allowing the body to move. These interfaces are particularly vulnerable to injury and tearing due to biomechanical inconsistency in the connections between soft and hard tissues. Therefore, engineering the musculoskeletal tissue interfaces continues to be a challenge [1]. In this project, I aim at developing polymeric fibrous scaffolds, mimicking native interfaces, with mechanical and biological performance influenced by the scaffold's design and topology. To produce fibrous scaffolds from polycaprolactone (PCL), 3D printing method called Melt Electrowriting (MEW) was used. Mechanical properties of scaffolds with different geometries and pore sizes were analyzed by tensile testing and modelled in computer simulation. Biological performance was investigated through cell attachment and proliferation on the produced scaffolds. Results indicated that the mechanical properties (Young's modulus, ultimate strength, maximum load) of the successfully fabricated scaffolds depend on the design structure. Biological evaluation showed that the cells attach, spread along the scaffolds and proliferate. Computer simulations helped to reveal the stress distribution in the produced scaffolds. In conclusions, MEW allows printing scaffolds with precise architecture. The mechanical properties and cell behavior can be controlled by design. In the next steps, gradient scaffolds will be produced. This study shows that MEW scaffolds have great potential in interface tissue regeneration.

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Abstract **#1751**

THE POTENCY OF JACKFRUIT SAP (ARTOCARPUS HETEROPHYLLUS) FOR HEALING RUPTURED TENDON IN RATS (RATTUS NORVEGICUS)

Fajar Shodiq Permata[P][1], Aditya Fernando[1], Aulia Dyasti Maurenda[1], Silvira Tri Purnama Sari[1], Akhmad Rifky Tribagus Rifandi[1], Edwin Widodo[2], Aulanni'am Aulanni'am[1]

[1] *Faculty of Veterinary Medicine, Universitas Brawijaya*

[2] *Faculty of Medicine, Universitas Brawijaya*

Corresponding author's email: drh.fajar@ub.ac.id

One of the most common health problems in racehorses is Achilles tendon rupture. If Achilles tendon ruptured, it would be difficult or even impossible for the horse to move their feet. Jackfruit sap (Artocarpus heterophyllus) is one of the natural alternative ingredients containing pectin that can act as an anti-inflammatory and has antibacterial substance and cellulose that can act as an adhesive compound. The research purposed to examine the potency of jackfruit sap for healing tendon rupture in the rat as the animal model. Left of Achilles tendon of rats were ruptured 50% using surgery then treated by 1 mg jackfruit sap. Rats have cared during the following 14 days. Nociceptive withdrawal time (NWT) was measured in day 15th then rats were euthanized, and tendon tissue was collected in formaldehyde 4%. Examination of tendon tissue consisted of histopathology, collagen density and thickness, inflammatory cells, expression of IL-1β, TGF-β and VEGF. Results showed jackfruit sap treatment could improve the healing of tendon after ruptured based on increasing NWT, improvement tendon tissue and formation of new collagen. However, the direction of the new collagen was irregular. Rats which given jackfruit sap have reduction IL-1β ($p<0.05$) and elevation ($p<0.05$) of TGF-β and VEGF. The conclusion was the jackfruit sap could be candidate material for bio-sealant to the ruptured tendon

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Abstract **#1753**

AEROSOL FORM OF DINITROSYL IRON COMPLEXES ACCELERATES WOUND HEALING

Aleksandra Butenko[P][1], Fayzullin Alexey[1], Semyon Churbanov[2], Natalia Serejnikova[1], Natalia Chepelova[1], Polina Shevchenko[1], Karen Mikaelyan[1], Anatoly Shekhter[1], Anatoly Vanin[2]

[1] *Sechenov First Moscow State Medical University (Sechenov University)*

[2] *Semenov Federal Research Center of Chemical Physics, Russian Academy of Sciences*

Corresponding author's email: butenko.aleksa@mail.ru

Dinitrosyl iron complexes (DNIC) are endogenous donors of nitric oxide. For more than 15 years, they have been studied to stimulate regeneration. However, the most effective dose and delivery form have not been defined yet. The aim of our research was to develop the aerosol with DNIC and study its effects on regeneration.

We developed a series of aerosols containing DNIC and modeled planar full-thickness wounds in Wistar rats. There were 4 groups of 6 animals. In the control group, the wounds were irrigated with distilled water. In study groups, the wounds were treated with aerosols containing 10, 50 and 100 μg of DNIC. Wound tissues were excised 4 days after the operation and analyzed by histological and morphometric methods.

DNIC aerosol with the dose of 50 μg had the most beneficial pro-regenerative effect on wound healing. In this group, the granulation tissue layer was 140% thicker, the intensity of inflammation was significantly lower than in the control ($p<0,05$). Aerosol with 10 μg of DNIC stimulated cell proliferation and growth of granulation tissue, but it did not facilitate it's the maturity and had no anti-inflammatory effect. Aerosol with 100 μg of DNIC increased the activity of inflammation, and had a less effect on the thickness and maturity of granulation tissue than the aerosol with 50 μg of DNIC. We demonstrated a dose-dependent decrease in the functional activity of mast cells in the experimental groups compared to the control.

DNIC aerosol is an effective delivery form for the treatment of wounds.

Keywords

Wound healing; Nitric Oxide; Dinitrosyl iron complex

Abstract **#1754**

THE INFLUENCE OF MATRIX ELONGATION ON DRUG RELEASE FROM PACLITAXEL ENRICHED 3D MATRICES PRODUCED BY ELECTROSPINNING

Zhanna Nazarkina[1], Boris Chelobanov[1], Irina Romanova[1], Pavel Laktionov[1,2]

[1] *Institute of Chemical Biology and Fundamental Medicine, SB RAS*

[2] *Meshalkin National Medical Research Center, Ministry of Health RF*

Corresponding author's email: zha_naz@niboch.nsc.ru

Paclitaxel (PTX)-eluting coating of vascular stent prevent neointima growth after stenoticarteries stenting. Matrices produced from 5%PCL/10%HSA/3%DMSO/PTX by electrospinning were shown to be suitable for stent coating as exhibiting long-term PTX release kinetic [1, 2]. Installation of vascular stents considers two-fold increase in stent diameter and, therefore, elongation of the matrices covering the stents. We studied the influence of matrix elongation on its structure and PTX release.

Three different matrices (5%PCL/PTX, 5%PCL/10%HSA/PTX and 5%PCL/10%HSA/3%DMSO/PTX) were prepared using electrospinning on a drum collector. Matrices were fixed between clamps and uniaxially elongated in 2 times and discs cut from relaxed matrcies were incubated for different time intervals (up to 27 days) in PBS or in human blood plasma to evaluate tritium-labeled PTX release in supernatants.

According to SEM data, all matrices were composed of randomly oriented fibers with smooth surface. Matrix elongation results in unequal deformation accompanied by fibers orientation along the applied force vector; breaks and changing the fiber diameter were not observed. Modelling of matrix deformation showed that thickness and specific PTX content do not significantly depend on discs positioning. The elongation has practically no effect on the kinetics and completeness of drug release from matrices.

The data obtained demonstrate that a matrix elongation during stent installation does not lead to fiber breaks and does not interfere with kinetics of PTX release.

Keywords

drug-eluting stents; Paclitaxel; electrospinning

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Abstract **#1755**

BIODEGRADABLE SMALL-DIAMETER VASCULAR GRAFTS MODIFIED WITH ILOPROST AND 1,5-BIS-(4-TETRADECYL-1,4-DIAZONIABICYCLO[2.2.2]OCTAN-1-YL) PENTANE TETRABROMIDE ARE RESISTANT TO THROMBOSIS AND INFECTION

Larisa V Antonova$_P$[1], Evgeniya O Krivkina[1], Olga V Gruzdeva[1], Maria A Rezvova[1], Vladimir N Silnikov[2], Tatiana N Akentyeva[1], Leonid S Barbarash[1]

[1] *Research Institute for Complex Issues of Cardiovascular Diseas, Kemerovo, Russian Federationes*

[2] *Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federationes*

Corresponding author's email: antonova.la@mail.ru

Development of thrombosis- and infection-resistant vascular prostheses is still an unmet clinical need. Here we designed a biodegradable vascular drug-eluting graft fabricated from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(ε-caprolactone) and modified with iloprost and cationic amphiphile 1,5-Bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl) pentane tetrabromide (0.25, 0.5 and 1 mg/mL). The surface modification was performed through complexation with polyvinylpyrrolidone, which was polymerized with the luminal surface by means of 15 kGy ionizing radiation. We further evaluated mechanical properties, hemocompatibility, and resistance to Klebsiella pneumonia spp. ozaena No. 5055, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Proteus mirabillis ATCC3177, and Pseudomonas aeruginosa. Ionizing radiation and polymerization of polyvinylpyrrolidone did not affect the mechanical competence of the graft. Iloprost/1,5-Bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl) pentane tetrabromide coating nullified the thrombogenicity of the luminal surface, reducing platelet aggregation by 5-7-fold, and efficiently prevented the growth of microbial pathogens. However, 1,5-Bis-(4-tetradecyl-1,4-diazoniabicyclo[2.2.2]octan-1-yl) pentane at a concentration of 1 mg/mL provoked considerable hemolysis and platelet aggregation. This study was funded by Russian Science Foundation, grant number №20-15-00075.

Abstract **#1756**

NOVEL PERFUSABLE MICRO ENGINEERED VASCULAR 3D-BIOPRINTED TUMOR MODEL FOR DRUG SCREENING

Lena Neufeld$_P$[1], Noa Reisman[1], Yael Shtilerman[1], Eilam Yeini[1], Ronit Satchi-Fainaro[1]

[1] *Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Israel*

Corresponding author's email: lenaneufeld246@gmail.com

Many drugs show promising results in laboratory research, but eventually fail in clinical trials. We hypothesize that one main reason for this translational gap is that the cancer models used are inadequate. Most models lack the tumor-stromal cells interactions with their microenvironment which are required for tumor progression. Such interactions do not form in conventional 2D cultures where cells grow on rigid plastic plates. Therefore, there is a need to develop a 3D model that better mimics the tumor microenvironment. Here, we recapitulated the tumor heterogenic microenvironment by creating fibrin GB-bioink consisting of patient-derived glioblastoma cells with astrocytes and microglia. Additionally, perfusable blood vessels were created with sacrificial bioink covered with brain pericytes and endothelial cells. We observed similar growth curves, drug response and genetic signature of glioblastoma cells grown in our unique 3D-bioink platform and in in-vivo studies as opposed to 2D culture. This 3D-bioprinted model could be the basis for potentially replacing cell cultures and animal models as a powerful platform for rapid, reproducible and robust personalized therapy screen and drug development.

Abstract **#1757**

PENETRATION OF SIROLIMUS AND PACLITAXEL RELEASED BY ELECTROSPUN PRODUCED DRUG ELUTING STENTS COATINGS THROUGH THE ARTERIAL WALL

Zhanna Nazarkina[1], Boris Chelobanov[1], Konstantin Kuznetsov[1,2], Irina Romanova[1], Tatyana Savostyanova[1,3], Pavel Laktionov[1,4]

[1] *Institute of Chemical Biology and Fundamental Medicine, SB RAS*

[2] *City Clinical Hospital no. 1, Novosibirsk, Russia*

[3] *Novosibirsk National Research State University, Novosibirsk, Russia*

[4] *Meshalkin National Medical Research Center, Ministry of Health RF*

Corresponding author's email: zha_naz@niboch.nsc.ru

Drug-eluting stents (DES) were designed to minimize neointima growth after angioplasty [1]. Sirolimus (SRL) and paclitaxel (PTX) are the most commonly used DES coating drugs now. It was shown that, PCL-based electrospun produced, SRL and PTX enriched matrices exhibit long-term drug release kinetic and are to be used as coatings of DES [2-4]. The retention of drug released from stent coatings by the wall of rabbit iliac artery (IAW) was studied.

DES coated with matrices 5%PCL/10%HSA/3%DMSO/SRL or 5%PCL/10%HSA/3%DMSO/PTX with tritium-labeled drugs in dose of 0.9 and 0.46 μg/cm2, respectively, were produced using electrospinning. Coated stents were installed into freshly obtained rabbit's iliac artery and fixed in special device. The drug release and penetrating through IAW in PBS or in blood plasma (BP) was evaluated by radioactivity of PBS/BP.

IAW retain PTX and decrese release from coating in 3-4 times during first hours and 2-2.5 times during the day. After 24 hours more than half of PTX released from the coating is retained in IAW. Even more efficient retention was observed for SRL; only 12.5% of SRL released from coating into BP pass through the IAW. Retention of drugs by IAW is more efficient when drugs were released in BP with a plateau reached in 9 hours. The retention/accumulation of drugs by IAW provides a prolonged drug release and allows reducing the dose of drugs in stent coatings.

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Keywords

drug-eluting stents; paclitaxel; sirolimus

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Abstract **#1758**

CROSS-LINKING OF MULTILAYER SYSTEMS FROM ALGINATE AND CHITOSAN WITH METAL IONS – EFFECTS ON ADHESION, PROLIFERATION AND ADIPOGENIC DIFFERENTIATION OF MULTIPOTENT MOUSE FIBROBLASTS

Husian Kindi[P][1], Thomas Groth[1,2], Christian Schmelzer[3], Matthias Menzel[3]

[1] *Department Biomedical Materials, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Heinrich-Damerow-Strasse 4, 06120 Halle (Saale), Germany.*

[2] *Interdisciplinary Center of Materials Science, Martin Luther University Halle-Wittenberg, 06120 Halle (Saale), Germany.*

[3] *Biological and Macromolecular Materials Business Unit, Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Halle (Saale), Germany*

Corresponding author's email: husnia.kindi@student.uni-halle.de

Bioactivity of metal ions influences the behaviour of cells like proliferation and differentiation. Here metal ions (Ca2+, Co2+, Cu2+ and Fe3+) were used for cross-linking of chitosan (Chi) and Alginate (Alg) polyelectrolyte multilayers (PEM) by coordination-based intrinsic cross-linking of functional groups like amino and hydroxyl groups. Physicochemical properties of PEM were investigated to characterize layer growth and thickness. Further the quantity of metal ions within multilayers was determined using ICP-MS. The effect of metal ions on surface properties was investigated such as wettability, mechanical properties and topography. The findings indicate that changes in physical properties depend on the type and concentration of metal ion. The adhesion and proliferation studies with murine C3H10T1/2 embryonic fibroblasts demonstrated that coordination-based crosslinking of Chi/Alg PEM with of metal ions had no adverse effect on cell behaviour. Interestingly, PEM cross-linked by copper and iron ions enhanced not only adhesion and proliferation of cells, but induced cell differentiation towards adipocytes. These findings indicate that metal ion-loaded PEM have the potential to guide stem cell differentiation on surfaces of implants and tissue engineering scaffolds.

Keywords:
Chitosan, alginic acid, intrinsic cross-linking, metal ions, cell adhesion, adipogenic differentiation.

Abstract **#1759**

TRANSPLANTATION OF AUTOLOGOUS ORAL MUCOSAL EPITHELIAL CELL SHEETS INHIBITS THE DEVELOPMENT OF ACQUIRED EXTERNAL AUDITORY CANAL ATRESIA IN A RABBIT MODEL

TARO INAGAKI[P][1]

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Corresponding author's email: t2inagaki@marianna-u.ac.jp

External auditory canal atresia (EAC) is frequently encountered in clinical practice. Causes of the condition are physical stress, trauma, infection, and ear surgery. Surgery is the treatment of choice; however, is associated with high rate of recurrence [1]. In this study, we artificially created EAC in a rabbit model. We also transplanted oral mucosal cell sheets, which were cultured on temperature responsive inserts, immediately after exfoliating the skin of the external auditory canal to prevent from atresia.
The aim of this study was to explore the possibility of a new therapeutic modality for EAC, and evaluate its effectiveness.

Keywords
external ear canal; atresia

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Abstract **#1760**

A SPHEROID MODEL OF EARLY- AND LATE-STAGE OSTEOSARCOMA HIGHLIGHTS THE DIVERGENT RELATIONSHIP BETWEEN TUMOR ELIMINATION AND BONE REGENERATION

Fiona Freeman[1,2,3], Ross Burdis[1], Daniel Kelly[1], Natalie Artzi[2,3]

[1] *Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland*

[2] *Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.*

[3] *Department of Medicine, Division of Engineering in Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA.*

Corresponding author's email: freemanf@tcd.ie

Osteosarcoma is the most diagnosed bone tumour that occurs in children. The use of tissue engineering strategies after malignant tumour resection remains a subject of scientific controversy as standard tissue engineering tools might exert tumour-promoting effects. As a result, there is limited research that focuses on bone regeneration post-resection, which is further compromised following chemotherapy. Due to the increasing interest in developing 3D-culture systems to provide unique means for screening therapies pre-clinical trials, this study aims to develop the first co-culture spheroid model for osteosarcoma to understand the divergent relationship between tumour elimination and bone regeneration. By manipulating the ratio of stromal to osteosarcoma cells the cancer state (early vs. late) was modified, as evident by increased tumour growth rates and altered ALP and VEGF expression levels, which are known clinical prognostic osteosarcoma factors[1,2]. When treated with BMP-2 or PDGF our model indicates, as expected, that osteogenic supplements significantly increase tumour growth in early-stage osteosarcoma. However, osteogenic supplements had no effect on tumour growth when applied to the late-stage osteosarcoma model. Regardless of cancer state, treatment with PDGF significantly increased the release of VEGF, and both osteogenic supplements significantly enhanced mineral formation within the tumour. When treated with chemotherapeutics in combination with osteogenic supplements, both tumour growth and mineral formation was significantly diminished, as previously seen clinically. Using our model, we can begin to understand mechanistically the relationship between stromal and osteosarcoma cells and their response to chemotherapeutics and regenerative cues outcomes that can inform the design of future therapies.

Keywords

Osteosarcoma; Organoid; Disease model

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Abstract **#1761**

THE X-SERIES: AN ECM BASED BIOINKS FOR PRINTING COMPLEX INVITRO TISSUE MODELS

Himjyot Jaiswal[1], Volodymyr Kuzmenko[1], Josefin Blell[1], Elin Pernevik[1], Itedale Namro Redwan[1]

[1] *Cellink*

Corresponding author's email: ep@cellink.com

3D bioprinting is changing the way in vitro models are generated to study cell behaviour, tissue properties and biology. The technique is used to fabricate 3D constructs layer-by-layer using living cells with biomaterials. Various biomaterials are utilized to develop bioinks which in return are used to fabricate constructs with biomimetic microenvironment and mechanical properties that are similar to native tissue. In recent years, the development of appropriate bioinks for tissue fabrication has drawn attention towards the decellularized extracellular matrix (dECM). The ECM composition and structure are unique to each tissue and cells and is impossible to replicate using native and synthetic polymers. Despite their excellent biochemical and functional properties, ECM does not possess sufficient mechanical strength to fabricate 3D structures that can retain its shape postprinting. To overcome this hurdle, CELLINK has launched the X-series that uses proprietary Nanocellulose to formulate an ECM containing bioink with improved mechanical properties to bioprint complex 3D structures. The first in the X-series is HEP X, a liver ECM based bioink. Structures printed using HEP X maintained shape and stability throughout 21 days of culture. HepG2 cells biofabricated using HEP X showed high cell viability with expression of liver specific metabolic markers e.g., albumin, alpha-fetoprotein, and E-cadherin. Not surprisingly, expression of albumin and serpina1 increased over time whereas alpha-fetoprotein expression decreased demonstrating that ECM enables liver specific function close to in vivo. Thus, dECM based bioinks are excellent candidates for insitu bioprinting for various applications due to their superior mechanical stability and biochemical properties

Abstract **#1762**

BIPHASIC TISSUE-SPECIFIC EXTRACELLULAR MATRIX DERIVED SCAFFOLDS PROMOTE ZONALLY DEFINED ARTICULAR CARTILAGE REGENERATION IN A CAPRINE OSTEOCHONDRAL DEFECT MODEL

David Browe[P][1], Pedro Diaz Payno[1], Rossana Schipani[1], Fiona Freeman[1], Ross Burdis[1], Daniel Ahern[1], Lindsey Randell[1], Selcan Guler[1], Jessica Nulty[1], Conor Buckey[1], Pieter Brama[2], Daniel Kelly[1]

[1] *Trinity College Dublin*

[2] *University College Dublin*

Corresponding author's email: browed@tcd.ie

Cartilage defects in which the subchondral bone is exposed require surgical intervention to induce repair in order to prevent further degeneration of the joint. The complex depth-dependent structure of articular cartilage (AC), consisting of an arcade-like collagen fibre network, enables it to withstand and distribute the challenging biomechanical loads passing through the joint. While some clinical advances in cartilage repair have occurred, osteochondral (OC) defect repair still remains a significant clinical challenge, with current scaffold and tissue engineering based approaches failing to recapitulate the complex, hierarchical structure of native AC. To address this need, we have developed a bi-phasic extracellular matrix (ECM)-derived scaffold fabricated from solubilized AC and bone. In vitro, porous scaffolds generated from these ECM components were shown to preferentially direct the differentiation of mesenchymal stromal cells (MSCs) towards a chondrogenic or osteogenic lineage depending on the ECM source. In addition, to further enhance tissue deposition and collagen fibre alignment, a novel freeze-drying fabrication method was developed to introduce pore alignment into the scaffolds. Finally, we implanted the scaffolds as an off-the-shelf, cell-free implant into a clinically relevant large animal model (caprine) of OC defect repair. Evaluation at 6 months post-implantation demonstrated that treatment of OC defects with the bi-phasic scaffold enhanced cartilage matrix deposition when compared to empty controls. Furthermore, the structure and composition of the repair tissue in scaffold treated defects was more hyaline-like in nature, consisting of a superficial zone rich in lubricin with aligned collagen fibres.

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Abstract **#1763**

SEMI-IPN PHBV/PVA NANOCOMPOSITE HYDROGELS WITH CONDUCTIVE NANOPARTICLES FOR TISSUE ENGINEERING

Jose Luis Aparicio Collado[P][1], Juan José Novoa[2], José Molina Mateo[1], Constantino Torregrosa Cabanilles[1], Ángel Serrano Aroca[3], Roser Sabater i Serra[1,4]

[1] Centre for Biomaterials and Tissue Engineering, Universitat Politècnica de València, 46022 València, Spain

[2] University of Applied Sciences Technikum Wien, Vienna, Austria

[3] Biomaterials and Bioengineering Lab, Centro de Investigación Traslacional San Alberto Magno, Universidad Católica de Valencia San Vicente Mártir, 46001 Valencia, Spain

[4] CIBER-BBN, Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine, 46022 València, Spain.

Corresponding author's email: joapcol@upvnet.upv.es

Tissue engineering (TE) combines both cells' biology and engineering to develop bioactive biomaterials to treat tissue regeneration [1]. Among them, biomaterials with conducting properties have shown promising potential as bioactive cell substrates for tissue regeneration.

Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is a biocompatible and biodegradable material widely employed in the biomedical field. It has a hydrophobic behavior and excellent mechanical properties. However, it is brittle, highly hydrophobic and shows poor thermostability [2]. Thus, the combination of PHBV with another hydrophilic biomaterial such as poly(vinyl alcohol) (PVA), a synthetic, hydrophilic and biocompatible polymer could be an interesting approach [3]. Additionally, conducting polymers such as polypyrrole (PPy) have attracted great interest in the biomedical field due to its ability to stimulate cell response [4]. Therefore, PHBV/PVA semi-interpenetrating networks (semi-IPN) with electroactive properties can be an interesting approach for TE applications.

Here we describe a novel semi-IPN composed by PHBV and crosslinked PVA in a 30:70 w/w ratio to conform an electroactive hydrophilic network with incorporated conductive polypyrrole nanoparticles (PPy) (up to 15%) [2]. Physicochemical properties have been analyzed by FTIR, DSC, TGA, swelling, water contact angle and dielectric spectroscopy. Electronic microscopy was used to perform a structural analysis.

PHBV/PVA/PPy semi-IPNs present good miscibility, no phase separation and strong interactions between the components. The hybrid hydrogels show increased conductivity with higher content of PPy nanoparticles and higher thermal stability compared to neat components. These properties postulate these nanocomposite hydrogels as promising biodegradable biomaterials for TE applications.

Acknowledgments

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Keywords

Semi-Interpenetrating Network; Conductive Composite Hydrogel ; Polyhydroxybutyrate-co-Hydroxyvalerate

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Abstract **#1764**

MICROFLUIDIC 3D BIOPRINTING OF A BLOOD BRAIN BARRIER AS A BIOMIMETIC PLATFORM FOR DRUG DELIVERY

Chiara Scognamiglio$_P$[1]

[1] *Istituto Italiano di Tecnologia*

Corresponding author's email: chiara.scognamiglio@iit.it

The majority of cerebrovascular diseases is connected to blood brain barrier (BBB) dysfunctions (1). Their treatment constitutes a major challenge due to the intrinsic properties of the BBB, which strictly controls the passage of molecules from the blood towards the central nervous system (CNS), making inefficient therapeutic drugs delivery (2). Recent studies have highlighted the potential of microfluidics and 3D bioprinting techniques for in vitro models of human blood vessels (3, 4).

We propose a biomimetic 3D printed BBB that provides an innovative platform to assess brain endothelial permeability for disease modeling and drug delivery applications. The microfluidic printhead mounted on a custom made extrusion bioprinter allowed for a continuous and precise deposition of multicellular and multimaterial inks. A blend of alginate and gelatin methacryloyl was used to fabricate a hollow fiber containing human brain-like endothelial cells (hBLECs) and bovine pericytes. We demonstrated the validity of the bioprinted model, checking for cells viability and staining f-actin and anti-zonula occludens-1 (ZO-1) to visualize the actin cytoskeleton and the tight junction at the cell-cell contact. In the presence of astrocytes conditioned medium and shear stress imposed by the flow, cells exhibited BBB phenotypes, among which flow-dependent cell alignment and tight junction formation. The tightness of the barrier is quantified through measurement of the fluorescent dye (40 kDa dextran) diffusion across the endothelial layer as a function of the shear stress exerted on the lumen. We thus show the importance of recreating a physiological shear stress to obtain a stable and tight BBB.

Keywords

Biofabrication; Microfluidic 3D bioprinting; Blood brain barrier

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Abstract **#1765**

INTRA-ARTICULAR INJECTION OF MESENCHYMAL STROMAL CELL ENCAPSULATED IN MICRO-MOLDED ALGINATE PARTICLES FOR THE TREATMENT OF POST-TRAUMATIC KNEE OSTEOARTHRISTIS IN RABBIT

Fabien NATIVELP[1,2,3], Audrey SMITH[1], Mélanie MARQUIS[4], Denis RENARD[5], Olivier GAUTHIER[6], Claire VINATIER[1,7], Anne des RIEUX[8], Jerome GUICHEUX[1,9], Catherine LE VISAGE[1]

[1] *Inserm, UMR 1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS Nantes*

[2] *CHU Nantes, Pharmacie Centrale, PHU 11*

[3] *UFR Sciences Pharmaceutiques et Biologiques, Université de Nantes*

[4] *UMR0703 PAnTher (Physiopathologie Animale et BioThérapie du muscle et du système nerveux), INRAE, ONIRIS*

[5] *UR1268 BIA (Biopolymères Interactions Assemblages), INRAE*

[6] *ONIRIS Nantes-Atlantic College of Veterinary Medicine, Centre de recherche et d'investigation préclinique (CRIP)*

[7] *UFR Odontologie, Université de Nantes*

[8] *UCLouvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Brussels, Belgium*

[9] *CHU Nantes, PHU 4 OTONN*

Corresponding author's email: fabien.nativel@univ-nantes.fr

Osteoarthritis (OA) is the most common inflammatory and degenerative joint disease. Mesenchymal Stromal Cells (MSCs), with their immune-regulatory properties and chondro-protective effects, have been considered as a new approach to treat OA. Considering the risk of cell leakage and death after intra-articular injection, we hypothesized that cell encapsulation in cytoprotective hydrogels could overcome these limitations and provide cells with a suitable 3D microenvironment supporting their biological activity. We have generated micromolded alginate microparticles (diameter 150 µm) and demonstrated the long-term viability of microencapsulated human adipose stromal cells (hASCs), as well as the morphological and mechanical stability of the particles. Encapsulated cells maintained their in vitro ability to sense and respond to a pro-inflammatory environment (IFN-γ/TNF-α (20 ng/mL) or synovial fluids from OA patients) by secreting PGE2, IDO, HGF and TGF-β. We then evaluated their anti-OA efficacy in a post-traumatic OA model in adults rabbits (n=24). Eight weeks after anterior cruciate ligament transection, destabilized joints were injected (intraarticular, 26G needle) with 200 µL of either PBS, blank microparticles, non-encapsulated or microencapsulated cells (5x105 cells). Six weeks after injection, a reduced severity of OA lesions was observed in joints injected with hASCs, whether encapsulated or not, as demonstrated by OARSI scoring on safranin-O stained sections. In addition, a significantly decreased expression of the aggrecanase-generated catabolic neoepitope NITEGE, as compared to joints injected with PBS or blank microparticles, was obtained, confirming the therapeutic effect of encapsulated cells on the diseased joint.

Keywords

Inflammation; Cell therapy; Anti-OA factors

Abstract **#1766**

ALL-IN-ONE: ON-CHIP FABRICATION AND IN-FLOW BIOPRINTING OF PERFUSABLE CELL-LADEN MICROGEL CONSTRUCTS

Benjamin Reineke[P][1], Stephan Förster[1], Ilona Paullus[2], Jürgen Groll[2]

[1] *Jülich Centre for Neutron Science (JCNS-1/IBI-8), Forschungszentrum Jülich GmbH, 52428 Jülich*

[2] *Department of Functional Materials in Medicine and Dentistry and Bavarian Polymer Institute, University of Würzburg, Pleicherwall 2, 97070 Würzburg, Germany*

Corresponding author's email: B.reineke@fz-juelich.de

Bioprinting of larger tissues and organs presents a tremendous opportunity to challenge the status quo for medical treatments of various illnesses. Necessary for the successful implementation of bioprinting is improving cell survival during the printing process, where cells are subjected to significant shear forces. A possible way to improve cell viability and to expand the bioprinting window is the encapsulation of cells in hydrogels, which can be done using microfluidics (1). This also allows for tailored cell microenvironments and for protection of cells from the immune system. Highley et al. recently proposed the use of microgel bioinks to overcome additional limitations of conventional bioinks, such as poor nutrient/oxygen diffusion (2). However, a limiting factor for the use of microgel bioinks so far is the required combination of microfluidics producing only small quantities, followed by post-chip batch processing such as cross-linking, separation and bioprinting. Here we present a novel approach to this problem by combining on-chip microgel formation, in-flow crosslinking and direct bioprinting of cell-laden microgel constructs. Within this “all-in-one” approach, microgels are formed using a double flow-focusing design, where a 3D contoured narrowing allows for a precise control of achieved droplet sizes. By incorporating an UV-LED at 385 nm into the setup, cross-linking of the microgels is performed in-flow in the microtubing before the microgels are printed directly using a modified FDM printer, resulting in constructs with intrinsic microporosity. These constructs can be manufactured in perfusion chambers, where they can be perfused easily via their porosity and integrated perfusion channels.

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Abstract **#1767**

TISSUE ENGINEERING FROM SCIENCE TO CLINIC – PRECISE BIO CORNEA

Rajesh Kumar$_P$[1], Lior Rosenberg Belmaker[2], Ariel Eisenbach[2], Amos Eitan[2], Yishay Hayardeni[2]

[1] *Precise Bio Inc, Winston-Salem, NC 27101*

[2] *Precise Bio Ltd, Modi'in, Israel*

Corresponding author's email: rajesh.kumar@precise-bio.com

Bioengineering has made impressive strides in recent years, yet the commercialization of bio-fabricated tissues and organs is still a great challenge. Precise Bio has taken up the challenge and established an industry-oriented workflow for taking academic products into the market.

To achieve commercial biofabrication, we have developed a high-end laser-based bioprinting method, allowing the deposition of picoliter-size drops of viscous bioink s (100s cP) containing single cells at high density. This continuous laser-induced-forward-transfer (co-LIFT) technique can print multiple bioinks simultaneously at high speed with controlled drop-size and high (>95%) cell viability. The co-LIFT bioprinter is incorporated into a 3D platform combining additional bio-fabrication technologies. We present the development of first bio- fabricated corneal endothelial grafts that are currently in preclinical studies. Other ophthalmologic tissues are in development and this biofabrication platform can be adapted to other tissues and organs.

The biofabricated corneal grafts are indicated for the treatment of corneal endothelial cell dysfunction that leads to corneal edema and blindness. Human donor corneal endothelial cells are expanded under GMP compliant conditions and bioprinted onto an ultra-thin collagen scaffold (10µm) at high cell density (~2700 cells/mm2) while maintaining biological activity. These grafts are easily implantable and reduce corneal swelling in a rabbit model of corneal endothelial dysfunction. Importantly, one donor may produce up to a hundred corneal grafts, thus potentially alleviating the corneal shortage.

Precise-Bio demonstrates here the ability to produce functional corneal endothelial implants, which can be extended to biofabricate other tissues for upscaling towards commercialization.

Abstract **#1768**

DESIGN OF PHOTO-CROSSLINKABLE COLLOIDAL GELS AS BIO-INKS FOR 3D PRINTING OF TISSUE ENGINEERING CONSTRUCTS

Mani Diba[P][1,2], Gerry Koons[1,2], Matthew Bedell[1,2], Antonios Mikos[1,2]

[1] *Department of Bioengineering, Rice University, Houston, TX, USA*

[2] *NIH/NIBIB Center for Engineering Complex Tissues, USA*

Corresponding author's email: manidiba@gmail.com

Biomaterials-based approaches exhibit great promise for regeneration of musculoskeletal tissues [1]. For these strategies, 3D printing techniques can deliver unprecedented control over architecture and properties of biomaterial constructs when combined with innovative material design strategies. Among different biomaterial categories, hydrogels have especially received significant interest for use in 3D printing of tissue engineering constructs [2], as they mimic various aspects of natural extracellular matrix. Unlike conventional (hydro)gels, colloidal gels are entirely assembled from particulate building blocks, enabling their use as modular biomaterial platforms. Although colloidal gels made of polymeric nanoparticles are attractive injectable and self-healing systems for tissue regeneration [3-5], their use as bio-inks for extrusion-based printing is largely unexplored.

Here, we report the development of photo-crosslinkable colloidal gels composed of photo-reactive gelatin nanoparticles for use as bio-inks for extrusion-based 3D printing. These nanoparticles are stabilized with intraparticle covalent crosslinks, and also contain pendant methacryloyl groups. While non-covalent interactions between nanoparticles enable formation of colloidal inks that are printable at room temperature, UV-induced covalent interparticle crosslinks based on methacryloyl moieties significantly enhance mechanical properties of printed constructs. Additionally, the UV crosslinking modality enables remarkable control over swelling, degradation, and biomolecule release behavior of the constructs. Finally, by exploiting the mechanical properties of UV-crosslinked colloidal gels, 3D constructs can be designed with shape memory behavior, returning to their original programmed shape upon re-hydration. Accordingly, these novel colloidal inks exhibit great potential to serve as bio-inks for 3D printing of biomaterials with shape-morphing features for applications such as bone tissue engineering.

Keywords

Nanoparticles; Colloidal gels; 3D printing

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Abstract **#1769**

LEGO®-INSPIRED MICROPOROUS SCAFFOLDS FOR PERSONALISED MANDIBULAR BONE REPAIR

Luan Phelipe Hatt$_P$[1,2], Sylvie Wirth[1,2], Keith Thompson[1], Karen Mys[1], Dominic Gehweiler[1], David Eglin[1,3], Martin J. Stoddart[1], Angela R. Armiento[1]

[1] *AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland*

[2] *Institute for Biomechanics, ETH Zürich, 8093 Zürich, Switzerland*

[3] *Mines Saint-Etienne, Univ Lyon, Univ Jean Monnet, INSERM, U 1059 Sainbiose, Centre CIS, F-42023 Saint-Etienne, France*

Corresponding author's email: phelipe.hatt@aofoundation.org

3D-printed personalised scaffolds are an attractive approach for mandibular bone repair. The challenging loading environment of this site requires biomaterials with suitable mechanical resilience, which may be provided via the addition of flexible materials such as thermoplastic polyurethane (TPU). This work aims to create a 3D printable personalised scaffold with a configurable layered composition, enhanced mechanical properties and improved cell adhesion. Varying combinations of PLGA (20-30%) and β-TCP (20%) are mixed with or without TPU (0-30%) in ethylene carbonate (EC) to obtain a printable ink (RegenHu Discovery®). Following water-mediated EC removal, surface microporosity and cytotoxicity is assessed using SEM and CellTiter-Blue®, respectively (Figure1A). A 3D model of a mandibular defect is derived from CT scans, then sliced and modified with CAD to obtain LEGO®-like structures. The personalised scaffolds are printed as a series of layers incorporating an interlocking mechanism (Figure1B). Scaffolds with precise and interconnected filaments can be printed using 30% PLGA, 20% β-TCP and 15% TPU. Lower PLGA concentrations (≤20%) in the absence of TPU results in brittle scaffolds. NMR confirms complete EC removal and SEM images show surface microporosity, while no cytotoxicity is reported in 3T3 cells. Large scale personalised mandibular implants can be successfully printed and assembled. We propose a novel 3D printable ink for mandibular bone reconstruction as an alternative to ceramics. Ongoing tests aim to demonstrate that the combination of ink composition, printing strategy and LEGO®-like assembly may confer beneficial mechanical properties suitable for mandibular repair, enhanced cell adhesion and efficient seeding of biologics intraoperatively.

Keywords

3D printing; Mandibular bone repair; LEGO-inspired® scaffold

Abstract **#1771**

BIOMATERIALS-DRIVEN BONE REGENERATION ON A MICROFLUIDIC CHIP

Maria Gabriella Fois[P][1], Alexander Pandias Margaronis Guttenplan[1], Zeinab Tahmasebi Birgani[1], Hoon Suk Rho[1], Stefan Giselbrecht[1], Pamela Habibović[1], Roman Truckenmüller[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine*

Corresponding author's email: g.fois@maastrichtuniversity.nl

Bone loss represents a major clinical challenge, requiring efficient regenerative therapies. Autografts are considered as the optimal treatment, but their limited availability and associated clinical complications have led to the development of synthetic alternatives, including those made from calcium phosphate (CaP) ceramics [1]. Conventionally, testing new biomaterials for bone regeneration has relied on in vitro studies followed by animal testing [2]. Recent advancements in cell culture platforms such as organ-on-chips (OOCs) have introduced new in vitro strategies to predict in vivo microenvironment [3]. We propose an OOC model to mimic biomaterial-driven bone regeneration. We engineered a microfluidic chip mimicking bone and a bone defect site, which aims to study homing of human bone marrow-derived mesenchymal stromal cells (MSCs) from the "host bone bed" into the "defect site" in the presence of biomaterials. Microfluidic chips were fabricated in polydimethylsiloxane using a two-step soft-lithography process [4]. The chips comprised a central chamber with independent inlet/outlet, mimicking the bone defect site, and two adjacent host bone bed-mimicking channels at opposite sides of the center chamber separated from the same by lines of pillars with a distance between them of 25 µm. MSCs were seeded and cultured in Matrigel® in the side channels. Migration of the MSCs towards the "defect site"-like central chamber was observed overtime using light microscopy. CaP microparticles (average diameter: 38 µm) were homogenously dispersed inside the central chamber of the chip. Next, we plan to study osteogenic differentiation and matrix deposition of migrated MSCs.

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Abstract **#1772**

CUSTOMIZED GELMA-BASED BIOINK FOR ENHANCED CARTILAGE TISSUE DEVELOPMENT

Volodymyr Kuzmenko$_P$[1], Mickis Gullstrand[1], Elin Pernevik[1], Itedale Namro Redwan[1]
[1] *Cellink AB, Arvid Wallgrens Backe 20, 413 46 Gothenburg, Sweden*
Corresponding author's email: ep@cellink.com

In tissue engineering and regenerative medicine, one of the hardest challenges is to create a relevant physical and chemical microenvironment that promotes cell attachment, proliferation and differentiation in order to enable a tissue-specific extracellular matrix formation. In this work, we present a bioink GelXA CARTILAGE for 3D bioprinting with tuned biomechanical properties to stimulate chondrogenesis.First of all, main bioink components, such as GelMA, alginate and xanthan gum, facilitate high resolution bioprinting at ambient conditions and provide the mechanical versatility of crosslinking. The choice between crosslinking methods (a softer ionic crosslinking, medium photocrosslinking or a stiffer one that combines both) allows stiffness adjustment of the cellular microenvironment to resemble different cartilage tissue zones.Strong interaction between chondrocytes and a matrix biomaterial is essential for chondrogenesis — cartilage tissue development — because chondrocytes comprise only 5% of total tissue volume and do not rely on cell-cell interaction. To achieve this, our bioink contains the necessary cell-matrix interaction components: 1) hyaluronan, an essential component of a cartilage ECM, and 2) laminin 521, regulating intracellular signaling in chondrogenesis by promoting cell adhesion to ECM and proliferation. Cell studies with human chondrocytes from femoral head showed that GelXA CARTILAGE successfully support high cell viability, attachment, and elevated expression level of a cartilage-specific proteoglycan aggrecan that is necessary for osmotic resistance against compressive loads and is a perfect sign of a chondrogenesis process.

Keywords
GelMA; Cartilage tissue; Stiffness regulation

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Abstract **#1773**

HUMAN PLATELET LYSATE AS A XENOGENEIC-FREE ALTERNATIVE FOR FETAL BOVINE SERUM IN HUMAN IN VITRO BONE MODELS

Bregje W. M. de Wildt[1], Keita Ito[1], Sandra Hofmann[1]

[1] *Orthopaedic Biomechanics, Department of Biomedical Engineering and Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, Eindhoven, The Netherlands*

Corresponding author's email: b.w.m.d.wildt@tue.nl

Bone has multiple mechanical and metabolic functions that are maintained through lifelong remodeling by osteoclasts and osteoblasts. To study this process while addressing the principle of replacement, reduction and refinement of animal experiments (3Rs), human in vitro bone models are being developed. Despite increasing safety, scientific, and ethical concerns [1], fetal bovine serum (FBS), a nutritional medium supplement, is still routinely used in these models. To comply with the 3Rs and to improve the reproducibility of such in vitro models, xenogeneic-free medium supplements should be investigated [1]. Human platelet lysate (hPL) might be a good alternative as it has been shown to accelerate osteogenic differentiation of mesenchymal stromal cells and improve subsequent mineralization [2, 3]. However, for a human in vitro bone model, hPL should also be able to adequately induce osteoclastogenesis and stimulate subsequent resorption. Here, we compared supplementation with 10% FBS vs. 10%, 5%, and 2.5% hPL for osteoclast formation and resorption by monocytes isolated from human buffy coats (N = 3). After 21 days, monocytes cultured with hPL showed a homogeneous and typical morphology for osteoclast, released significantly more TRAP into the culture medium, and were better capable of resorbing an osteo-assay surface when compared to monocytes cultured with FBS. These effects were visible at all hPL concentrations. Hence, hPL should be considered as a good xenogeneic-free alternative to FBS for in vitro bone models, supporting both osteoblast and osteoclast differentiation.

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Abstract **#1774**

NOVEL BIOINK DESIGN FOR 3D BIOPRINTING OF HUMAN PLURIPOTENT STEM CELL DERIVED CORNEAL EPITHELIAL CELLS

Paula Puistola[P][1], Maija Kauppila[1], Heli Skottman[1], Anni Mörö[1]
[1] *Eye Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland*
Corresponding author's email: paula.puistola@tuni.fi

There is high demand for developing biofabrication techniques to produce native-like corneal 3D structures. Unlike conventional methods, 3D bioprinting enables layer-by-layer assembly of cell-laden bioinks [1]. The current challenge is the lack of printable, cell-laden bioinks. Here, human pluripotent stem cell derived limbal epithelial stem cells (hPSC-LESCs) were used for bioprinting corneal epithelium mimicking structures. A novel bioink for extrusion-based bioprinting of hPSC-LESCs was designed and optimized by combining human and recombinant extracellular matrix proteins. Photocrosslinking with ultraviolet (UV) light and protein thiolation were investigated as crosslinking strategies for increased stability. The UV light exposure and the printing substrate were optimized based on the biocompatibility and cellular response. Transmittance and rheological measurements were used to characterize the transparency and rheological properties of the bioink.

The designed bioink possessed shear thinning properties and demonstrated near excellent transparency (65-75%), according to the corneal transparency classification [2]. The UV exposure did not decrease the cell viability (> 88%), however, it increased the crosslinking density and material stiffness. Because softer bioink and MatrigelTM coating promoted higher hPSC-LESC proliferation and further differentiation, bioprinting without additional photocrosslinking on MatrigelTM coated printing substrate resulted in the most stratified epithelium. No significant difference was observed between the bioink with or without the thiolated protein in the rheological measurements.

This was the first study where the stratification of hPSC-LESCs was observed after extrusion-based 3D bioprinting. The novel bioink showed great potential for bioprinting corneal epithelium mimicking structures using hPSC-LESCs and hold great promise in ocular surface reconstruction.

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Abstract **#1775**

HIGH THROUGHPUT SCREENING OF POLYMERIC BIOMATERIALS FOR PERSONALISED 3D PRINTED TREATMENTS

Adja Touré[1], Lewis Hart[2], Laura Ruiz Cantu[1], Derek Irvine[1], Wayne Hayes[2], Ricky Wildman[1]

[1] *Centre for Additive Manufacturing, University of Nottingham*

[2] *Chemistry Department, University of Reading*

Corresponding author's email: adja.toure@nottingham.ac.uk

Three-Dimensional (3D) printing is an established manufacturing method(1). However, the use of 3D printing is limited in industry due to few materials available and the long development and optimisation process. A high throughput methodology has been developed allowing to quickly asses the printability and biocompatibility of a large library of materials(2,3). This method is adapted and used here, to screen a library of 64 PCL-based hyper-branched polymers and determine their suitability in articular cartilage regeneration applications.

To screen the polymer library, their printability (Z parameter) for inkjet printing was first assessed using a liquid handler, able to measure viscosity and surface. The high-throughput assessment of mechanical and biocompatible properties were enabled using a microarray strategy. Surface chemical characterisation was carried out using time-of-flight secondary ion mass spectrometry (ToF-SIMS), while localised mechanical properties (elastic moduli) were determined using atomic force microscopy (AFM). Combination of these techniques with cytotoxicity screening led to the selection of three suitable polymers for the targeted application.

Solubility in DMF and printability determination led to the selection of around 60% of the tested library. Subsequently, the polymer arrays were fabricated by contact printing using super hydrophilic/hydrophobic glass slides as substrate. ToF-Sims surface analysis ruled out any chemical contaminations and confirmed the deposition of the desired polymers. Assessment of the mechanical properties and cytotoxicity screening led to the selection of three materials. Preliminary printing essays were undertaken. The polymer library showed interesting mechanical properties matching human articular cartilage (2-7 GPa) and demonstrated the feasibility of manufacturing such scaffolds.

Keywords

3D printing ; High Throughput Screening; Personalised Treatments

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Abstract **#1776**

REPLACEMENT OF MATRIGEL BY HUMAN PLACENTA ECM FOR BIOPRINTING APPLICATIONS

Johanna Berg[P][1], Viola Röhrs[1], Johannes Hackethal[2], Jens Kurreck[1]

[1] *Technical University Berlin, Institute for Biotechnology, Berlin, Germany*

[2] *THT-Biomaterials, Vienna, Austria*

Corresponding author's email: johanna.berg@tu-berlin.de

Bioprinting is a popular technology to generate three-dimensional humanized tissue models, which facilitate human specific research and can help to reduce animal experiments or even replace them. However, cell laden printed biomaterials, called bioinks, often contain the animal derived extracellular matrix (ECM) analogue Matrigel (a). Although frequently used in a wide variety of applications, its xenogenic origin from murine tumor limits the therapeutical potential and relevance for human specific approaches. Due to its complex composition ECM obtained from human placenta has great potential as xenofree ECM analogue in bioprinting strategies (b,c). Here we printed liver cells (HepaRG) in an alginate/gelatin bioink reinforced with either 40 % human placenta ECM; 20 % Matrigel or without further protein supplementation and cultured them for 21 days. Viability measurements and expression of hepato-specific markers (albumin, CYP3A4) showed comparable results between cells printed in Matrigel or placenta ECM conditions. Immunohistochemical analysis showed differences in distribution and morphology of the cells. Cells printed in Matrigel formed less, but bigger clusters of cells whereas cells printed in placenta ECM formed more, though smaller homogenously distributed cell clusters. Interestingly, release of IL-8 initially was lower after printing HepaRG cells under Matrigel conditions and became higher during the cultivation period. Placenta ECM conditioned cells revealed a complementary IL-8 expression profile, with higher release after printing, which became lower during culture. Even though further analysis must be made, placenta ECM reinforced bioinks are a promising approach to bioprint translationally relevant, functional human tissue models.

Keywords

Human Placenta ECM; Bioprinting

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Abstract **#1777**

THE MECHANO-RESPONSE OF TISSUE-ENGINEERED HEPARAN-SULFATE DEFICIENT CARTILAGE: AN ANTI-APOPTOTIC EXPRESSION SIGNATURE AT ELEVATED PROTEOGLYCAN PRODUCTION

Matthias Gerstner$_P$[1], Ann-Christine Severmann[2], Andrea Vortkamp[2], Wiltrud Richter[1]

[1] *Research Centre for Experimental Orthopaedics, Orthopaedic University Hospital Heidelberg, Heidelberg, Germany*

[2] *Dept. of Developmental Biology, University of Duisburg-Essen, Essen, Germany*

Corresponding author's email: matthias.gerstner@med.uni-heidelberg.de

Purpose: Osteoarthritis (OA), a major cause of disability world-wide, still evades efficient pharmacological or cellular therapies. Severe cartilage degeneration precedes the loss of mobility and disabling pain perception in affected joints. Recent studies showed that heparan-sulfate deficiency (HS^-) protects cartilage from OA in animal models of joint-destabilization, but underlying mechanisms remained unknown (1-3). We aimed to clarify whether low HS-content alters the mechano-response of chondrocytes and to uncover pathways relevant for chondro-protection.

Methods: Tissue-engineered HS^--cartilage was generated from murine rib chondrocytes hypomorphic in HS-synthesizing Exostosin1 (Ext1). Chondrocytes matured in agarose over 2 weeks were exposed to cyclic unconfined compression in a bioreactor. The molecular loading-response was determined by transcriptome profiling, bioinformatic-data processing and qPCR/WB.

Results: During maturation, HS^--chondrocytes expressed 3-6% of wildtype Ext1-levels, raised Sox9, Col2a1 and Acan like controls but synthesized and deposited 50% more GAG/DNA. Their TGF-ß and FGF2-sensitivity was similar to wildtype but the BMP-response was enhanced according to Western Blotting. Loading similarly activated mechano-sensitive ERK and P38-signaling in wildtype and HS^--chondrocytes. Transcriptome analysis reflected regulation of locomotion as major load-induced biological process and similar stimulation of novel mechano-regulated genes Inhba, Timp1, Ngf, Fosl1, Dhrs9 and Itgα5 in both groups was confirmed. Intriguingly, only HS^--cartilage responded to loading by an expression signature related to negative regulation of apoptosis with the apoptosis-regulator Bnip3 being exclusively downregulated in HS^--cartilage.

Conclusion: HS-deficiency enabled an anti-apoptotic impact of loading and enhanced the BMP-sensitivity plus GAG-production of chondrocytes all of which may protect HS^--cartilage from mechano-induced erosion.

Keywords

Heparan Sulfate; Osteoarthritis; Tissue Engineering

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Abstract **#1778**

NON-INVASIVE METABOLIC ASSESSMENT OF HEALTHY NEURAL SPHEROIDS AND SPHEROIDS WITH PATHOLOGY

Alena Kashirina[P][1], Aleksandra Kashina[1], Erdem Dashinimaev[2], Ekaterina Vorotelyak[2], Alena Gavrina[1], Emil Kryukov[1], Vadim Elagin[1], Elena Zagaynova[1,3]

[1] *Institute of experimental oncology and biomedical technologies, Privolzhsky research medical university, Nizhny Novgorod, Russia*

[2] *Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia*

[3] *Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia*

Corresponding author's email: bystrova93@gmail.com

Neurospheres created from patient-specific NSCs (neural stem cells) are a promising tool for cell replacement therapy or neurodegenerative diseases modeling. It is known that brain diseases are accompanied by changes in many cellular parameters, including their metabolic activity. In our work, we studied the metabolic status of healthy NSCs and NSCs with Down syndrome cultivated in three-dimensional conditions. To solve this problem, an analysis of the redox ratio (FAD/NAD(P)H), fluorescence lifetimes and fluorescence lifetimes contributions free and bound forms of NAD(P)H was carried out using fluorescence microscopy and FLIM (Fluorescence Lifetime Imaging Microscopy). Evaluation of the redox ratio showed that the values of this parameter are higher in neurospheres created from healthy NSCs, with an average value of 0.13 ± 0.02, compared with neurospheres with Down's syndrome, where hererogenicity is observed for this parameter with an increase from the periphery to the center (0, 05 ± 0.01 and 0.07 ± 0.01, respectively). This indicates that neurospheres with Down's syndrome are characterized by a more glycolytic phenotype than healthy ones. In addition, we demonstrated that NSCs with Down syndrome in the 3D model are characterized by lower a2 values (27.18 ± 1.16) than healthy NSCs cultivated under identical conditions (32.33 ± 1.42), which indicates their more glycolytic status, which is consistent with the redox ratio data. Thus, the metabolic status, assessed by optical imaging methods, can be used as a parameter for non-invasive non-contrast assessment of cells in neurodegenerative diseases modeling.

Keywords

Neurospheres; Down's syndrome; metabolism

Abstract **#1779**

MATRIX METALLOPROTEINASE-RESPONSIVE PVA HYDROGEL WITH TUNABLE PROPERTIES REGULATES DERMAL FIBROBLAST BEHAVIOR

Ke Yang[P][1], Berna Neidhart[1], Kongchang Wei[2], Katharina Maniura[1], Markus Rottmar[1]

[1] *Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biointerfaces, CH-9014 St. Gallen, Switzerland*

[2] *Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biomimetic Membranes and Textiles, CH-9014 St. Gallen, Switzerland*

Corresponding author's email: ke.yang@empa.ch

Skin injuries due to trauma or disease can be healed by the body via a complex series of events but often require clinical intervention. However, current treatment options are frequently not sufficient and evaluation of new treatment concepts remains difficult due to ethical concerns regarding animal models or lack of control over the properties of collagen-based skin equivalents.1 In this work, we are therefore developing 3D skin equivalents based on a highly controllable polyvinyl alcohol (PVA) hydrogel system that is photo-crosslinked by different matrix metalloproteinase (MMP)-sensitive pep-tides (GP and VP).2 The hydrogels were characterized for mechanical properties, and the viability of human dermal fibroblasts as well as matrix secretion were assessed over the culture period of 28 days. Despite using peptides with different MMP-sensitivity, cell-free hydrogels crosslinked with the same peptide concentration shared similar storage moduli, while higher cell viability, enhanced cell spreading as well as higher collagen production was observed in VP gels. These results suggest that the mechanical properties of the PVA hydrogel system are tunable by peptide concentration but are independent on the peptide sequence , providing a versatile platform for research on the biological effects of peptides. The VP peptide, being proteolytically more sensitive to MMPs, facilitated a faster degradation of the hydrogels, which is essential to allow for cell proliferation and spreading within this 3D environment. Further exploration of keratinocyte-fibroblast interactions based on this PVA hydrogel platform with tunable cellular responsiveness and promoted matrix secretion will facilitate the development of 3D skin equivalents.

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Abstract **#1780**

DEVELOPMENT OF NOVEL EFFECTIVE 'OFF THE SHELF' GENE THERAPEUTICS FOR INCORPORATION INTO ADVANCED BIOINKS FOR CARTILAGE REPAIR

Donagh G O'Shea[P][1,2], Lia A Blokpoel Ferreras[1,2], James E Dixon[3], Caroline M Curtin[1,2], Fergal J O'Brien[1,2]

[1] Tissue Engineering Research Group, Department of Anatomy and Regenerative Medicine, RCSI University of Medicine and Health Sciences, Dublin, Ireland.

[2] Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland.

[3] Division of Regenerative Medicine & Cellular Therapies (RMCT), The University of Nottingham Biodiscovery Institute (BDI), School of Pharmacy, University Park, University of Nottingham, Nottingham, UK.

Corresponding author's email: DonaghOShea@rcsi.ie

Background: Critical-sized cartilage defects require therapeutic intervention to promote healing. In our lab, we use tissue engineering strategies to address this by delivering pro-chondrogenic nucleic acid-based therapeutic nanoparticles (NPs) using 3D-printed (3DP) bioinks and scaffolds(1-3). However, clinical translation of these products may prove challenging due to a short shelf-life. Thus, this study aims to investigate lyophilisation as a process to enable stable storage of therapeutic NPs as novel 'off-the-shelf' formulations for incorporation into 3DP bioinks.

Methods: NPs consisting of a glycosaminoglycan-enhanced transduction (GET) peptide non-viral delivery vector complexed with plasmid DNA (pDNA) encoding for the reporter Gaussia luciferase were formulated at increasing charge ratios (CRs - 6, 9, 12) of peptide to pDNA and lyophilised using two respective cycles - a short cycle (SC)(4) and long cycle (LC)(5). NP physiochemical properties were determined. Rat MSCs were then transfected with SC and LC lyophilised NPs (L-NPs) and compared against freshly complexed NPs (F-NPs). Transfection efficiency and cell metabolic activity were determined over 72hrs. Long-term stability studies over 6mnths are ongoing.

Results: There was no significant difference in NP size, polydispersity or charge across groups at each CR. Comparable levels of transfection efficiency were achieved with SC L-NPs, LC L-NPs and F-NPs at CR 6 and 9 with no significant difference in cell metabolic activity.

Conclusions: Lyophilisation shows promise in ensuring stable storage and functionality of therapeutic NPs, paving the way for formulating clinically translatable 'off-the-shelf' gene-activated bioinks and 3DP scaffolds for cartilage repair.

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Abstract **#1781**

THE INCORPORATION OF COLLAGEN TYPE II INTO COLLAGEN HYALURONATE SCAFFOLDS IMPROVES EARLY CARTILAGINOUS MATRIX QUALITY AND REDUCES FURTHER DIFFERENTIATION TOWARD ENDOCHONDRAL BONE FORMATION

Claudio Intini[1,2], Tom Hodgkinson[1,2], John P. Gleeson[3], Fergal J. O'Brien[1,2]

[1] *Tissue Engineering Research Group, Department of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland*

[2] *Advanced Materials and Bioengineering Research (AMBER) Centre, RCSI & TCD*

[3] *Fraunhofer Project Centre for Embedded Bioanalytical Systems, Dublin City University, Glasnevin, Dublin 9, Ireland.*

Corresponding author's email: claudiointini@rcsi.com

The ability to regenerate damaged cartilage capable of long-term performance in an active joint remains an unmet clinical challenge in cartilage tissue engineering. Biomimetic implants have shown potential to direct high-quality cartilage tissue formation and to modulate key hypertrophic tissue/cellular events associated to endochondral bone formation1. In particular, type I collagen-hyaluronate (CHyA) scaffolds have demonstrated significant chondrogenic regenerative capacity preclinically2 and clinically3. However, the potential of these implants to a chondro-instructive higher quality cartilage tissue regeneration can be enhanced. The aim of this work was to investigate the incorporation of type II collagen (Coll2) (a potential chondro-inductive component4) within type I collagen (Coll1) and CHyA scaffolds and to assess its role in developing high-quality and long-term cartilage tissue formation. Coll1 and CHyA scaffolds in presence or absence of Coll2 were manufactured and cultured in vitro with human mesenchymal stem cells (hMSCs) for 28 days. The incorporation of Coll2 to Coll1 and CHyA scaffolds improved an early cartilaginous tissue formation and reduced hypertrophic tissue/cellular events in cartilage formation. In particular, the incorporation of Coll2 significantly decreased the expression of type X collagen protein by the hMSCs compared to Coll1 and CHyA scaffolds. Moreover, the incorporation of Coll2 to Coll1 scaffolds in combination with hyaluronate (HyA) led Coll2-CHyA scaffolds to the greatest expression of type II collagen protein by the cells. In summary, the incorporation of Coll2 to CHyA scaffolds demonstrated to improve cartilage regeneration and to indicate Coll2-CHyA scaffolds as prospective biomimetic implants capable to regenerate damaged cartilage for long-term performances.

Keywords

Biomimetic biomaterial; Cartilage regeneration; Osteoarthritis

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Abstract **#1782**

ORIENTATED POLYCAPROLACTONE NANOFIBERS OVER-RIDE BARRIER FORMING ASTROCYTE-SCHWANN CELL INTERFACES AND SUPPORT NEURITIC OUTGROWTH INTO THE ASTROCYTIC TERRITORY IN VITRO

Pascal Achenbach[P][1,2], Laura Hillerbrand[3], José Gerardo-Nava[4], Axel Dievernich[5], Dorothee Hodde[1], Paul Dalton[3], Andrij Pich[4], Joachim Weis[1], Haktan Altinova[1,6,7], Gary Brook[1]

[1] *Institute of Neuropathology, RWTH Aachen University Hospital, Aachen, Germany*

[2] *Department of Neurology, RWTH Aachen University Hospital, Aachen, Germany*

[3] *Department of Functional Materials in Medicine and Dentistry, University Hospital Würzburg, Würzburg, Germany*

[4] *DWI – Leibniz Institute for Interactive Materials, Aachen, Germany*

[5] *Department of General, Visceral and Transplant Surgery, RWTH Aachen University Hospital, Aachen, Germany*

[6] *Department of Neurosurgery, RWTH Aachen University Hospital Aachen, Germany*

[7] *Police Headquarters Berlin, Medical Commission, Berlin, Germany*

Corresponding author's email: pascal.achenbach@rwth-aachen.de

Astrocytes (AC) and Schwann cells (SC) are mutually repulsive glial cell populations of the central and peripheral nervous system, respectively. They juxtapose at spinal nerve root entry zones or in areas of spinal cord following severe traumatic injuries, where they establish a barrier that allows regenerative axonal growth from the AC to the SC territory but not in the other direction. The confrontation assay reproduces some of these growth inhibitory cell-cell interactions in vitro and is frequently used to test the barrier-modifying properties of potential therapeutic agents. However, so far only moderate success has been achieved. Here, the effectiveness of highly orientated electrospun polycaprolactone (PCL) nanofibers in over-riding this barrier is demonstrated. The elastic band spacer (EBS) technique was used to set up confrontation assays between AC and SC in the absence or presence of PCL nanofibers. The interface established between AC and SC in the absence of nanofibers showed only minor cell intermingling and acted as effective barrier to neurites derived from early post-natal dorsal root ganglia attempting to cross from the SC into the AC territory. In the presence of nanofibers, however, AC and SC adopted highly orientated morphologies and intermingled extensively, blurring the otherwise defined interface. In consequence, substantial numbers of neurites were able to cross the interface and extend for long distances into the AC territory. This observation reflects the first demonstration of non-functionalized nanofibers providing a bioengineered nanotopography that can over-ride the mutually repulsive nature of these cells in a model of nervous tissue scarring.

Keywords

PCL nanofibers; Astrocyte-Schwann cell interface; Axon regeneration

Abstract **#1783**

MESENCHYMAL STEM CELL DERIVED EXTRACELLULAR VESICLES AS A TREATMENT FOR OSTEOARTHRITIS IN A RAT HIGH FAT DIET GROOVE MODEL

Kelly Warmink$_P$[1], Devin van Valkengoed[1], Sabine Versteeg[2], Niels Eijkelkamp[2], Harrie Weinans[1], Nicoline Korthagen[1,3], Magdalena Lorenowicz[4]

[1] *UMC Utrecht, Department of Orthopedics*

[2] *UMC Utrecht, Laboratory for Translational Immunology*

[3] *LUMC, Department of molecular epidemiology*

[4] *UMC Utrecht, Center for Molecular Medicine, Section Stem Cells*

Corresponding author's email: k.warmink@umcutrecht.nl

Mesenchymal stromal/stem cells (MSC) hold great promise as treatment for osteoarthritis (OA) due to their immunomodulatory and regenerative capacities. Growing evidence shows that part of these effects are induced by extracellular vesicles (EVs). Here, we investigated whether MSC-derived EVs have anti-inflammatory and pro-regenerative properties in a rat high fat diet model for OA.

36 male rats were fed a high fat diet for 24 weeks and received groove surgery in week 12 to induce OA. One week after surgery rats were treated with either PBS, 2 million MSCs or EVs derived from 2 million MSCs. Pain behavior was monitored during the study and uCT scans were made at week 0 and week 24. Knee joints were harvested for histology to determine the OARSI histopathological score for rats.

Pain behavior showed no difference between the three treatment groups. On uCT the mean osteophyte number was slightly lower in the MSC group and cartilage mineralizations were observed in 9 of 12 animals in the PBS and MSC group and in 5 of 12 animals in the MSC-EV group. Total mineralization diameter was lowest in the MSC-EV group, but not statistically significant. Histological evaluation is ongoing.

In conclusion, we see little significant differences between treatments on uCT or von Frey test. However, we observe a large spread in the MSC and MSC-EV treated groups, possibly indicating that individual animals are responding differently. Before solid conclusions can be drawn a complete picture of the joint damage including cartilage and synovium is needed.

Abstract **#1784**

A SIMPLE, ECONOMICAL AND STANDARDIZED IN VITRO CELL MIGRATION ASSAY PRESERVING EXTRACELLULAR MATRIX SUBSTRATE AND NANOFIBRE-DERIVED TOPOGRAPHICAL CUES

Pascal Achenbach[P][1,2], Inge Hambeukers[1], Anna Pierling[1], José Gerardo-Nava[3], Laura Hillerbrand[4], Antonio Sechi[5], Katharina Glücks[1], Paul Dalton[4], Axel Dievernich[6], Andrij Pich[3], Haktan Altinova[1,7,8], Gary Brook[1]

[1] *Institute of Neuropathology, RWTH Aachen University Hospital, Aachen, Germany*

[2] *Department of Neurology, RWTH Aachen University Hospital, Aachen, Germany*

[3] *DWI – Leibniz Institute for Interactive Materials, Aachen, Germany*

[4] *Department of Functional Materials in Medicine and Dentistry, University Hospital Würzburg, Würzburg, Germany*

[5] *Institute of Biomedical Engineering, Department of Cell Biology, RWTH Aachen University, Aachen, Germany*

[6] *Department of General, Visceral and Transplant Surgery, RWTH Aachen University Hospital, Aachen, Germany*

[7] *Department of Neurosurgery, RWTH Aachen University Hospital, Aachen, Germany*

[8] *Police Headquarters Berlin, Medical Commission, Berlin, Germany*

Corresponding author's email: pascal.achenbach@rwth-aachen.de

It is widely accepted that the molecular composition of the extracellular matrix (ECM) and its inherent topographical cues influence cell migration in important processes such as wound healing and functional tissue regeneration. Current tissue engineering strategies for the repair of neurotmesis-type injuries of the peripheral nervous system (PNS) include directional cues (e.g. micro- or nanofibers) for orchestrating efficient regenerative growth. Further developments in such bioengineered materials require the use of quantifiable in vitro assays that are suitable for demonstrating the effectiveness of changes in ECM composition and/or application of topographical cues. Most popular in vitro migration assays monitor cell movement into a well-defined cell exclusion zone (CEZ) but are either not ideally suited or have not been tested for their ability to preserve the ECM substrate or its topographical features. The present investigation introduces the elastic band spacer (EBS) technique, an easy to use, economical and highly standardized migration assay based on the use of commercially available elastic bands for the generation of well-defined CEZs. The EBS technique preserves the ECM substrate of poly-L-lysine/fibronectin-coated glass coverslips as well as 2.5D nanotopography provided by arrays of adherent electrospun polycaprolactone (PCL) nanofibers whereas commercial adhesive silicon inserts and the popular scratch assay cause substantial disruption of the substrate. Application of the EBS approach in CEZ assays in the absence or presence of PCL nanofibres was validated monitoring the migratory behaviour of perineurial cells and Schwann cells, both of which are known to play important roles in PNS regeneration.

Keywords

Migration assay; ECM substrate; PCL nanofibers

Abstract **#1785**

LINKAGE OF BMP-2 AND NANOPARTICLES FOR ENHANCED BONE REGENERATION: BMP-2 DOSE FINDING AND BIOCOMPATIBILITY TESTING OF NANOPARTICLES

Lena-Christin Ingwersen[P][1], Doris Hansmann[1], Rainer Bader[1], Anika Jonitz-Heincke[1]

[1] *Biomechanics and Implant Technology Research Laboratory, Department of Orthopaedics, University Medical Center Rostock, 18057 Rostock, Germany*

Corresponding author's email: lena-christin.ingwersen@med.uni-rostock.de

Bone morphogenetic protein (BMP-)2 promotes osteogenic differentiation [1] whereas this can mainly be observed in clinical trials for doses in the milligram range [2]. Tissue engineering strategies aim for carrier systems for BMP-2 delivery to reduce negative side effects of high BMP-2 doses like ectopic growth and carcinogenicity [2,3]. The aim was to find appropriate BMP-2 doses and nanoparticles with a high biocompatibility for linkage and a controlled BMP-2 release.
The effect of BMP-2 (0.1, 1 or 10 µg/ml) was tested in human osteoblasts (n=6) for up to seven days. Metabolic and alkaline phosphatase (ALP) activities and gene expression of collagen 1 (COL1A1), ALP, BMP-2 and osteopontin (OP) were analyzed. In addition, the biocompatibility of silica and PLA nanoparticles (both: 500 nm diameter) in osteoblasts was determined via gene expression analysis, ALP activity and interleukin (IL)6 and 8 protein quantification.
Mainly exposure to 10 µg/ml BMP-2 resulted in significantly decreased metabolic ($p=0.012$) and increased ALP activity ($p= 0.012$). COL1A1 expression was decreased and ALP-, BMP-2- and OP-mRNA (all: $p=0.028$) were increased. While PLA nanoparticle biocompatibility analysis showed only a slight effect on gene expression and protein secretion in osteoblasts, silica nanoparticles induced a strong immune response at both gene expression and protein level with respect to IL6 and IL8 secretion.
Due to the good biocompatibility of PLA nanoparticles and the osteoinductive effect of low dose BMP-2, these parameters will be used for linkage to ensure a controlled release which will be evaluated in further studies.

Keywords

BMP-2; osteogenic differentiation; nanoparticles

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Abstract **#1786**

DESIGNING AN IN VITRO ANGIOGENESIS MODEL FROM PORCINE ARTERIAL ECM HYDROGEL FOR LONG-TERM HIGH THROUGHPUT RESEARCH

Tzila Davidovp[1], Marcelle Machluf[1]

[1] *Technion - Biotechnology and Food Engineering*

Corresponding author's email: tzila937@campus.technion.ac.il

Angiogenesis is a developmental process that plays a fundamental role in tissue development and different abnormalities and is, therefore, extensively studied worldwide to provide deep understandings of its ruling mechanisms. Low-cost high throughput research of angiogenesis is applied in In vitro models that offer the benefit of the use of human cells while sparing animal lives. Nevertheless, only temporal vessel formation is available in current in vitro models and, consequently, limited to a few days' assays. Considering these limitations, this study examines the hypothesis in which the mimicking of the physiological artery microenvironment can provide longer and more reliable angiogenesis processes in vitro. To this end, the development of a porcine arterial extracellular matrix (paECM) hydrogel is presented, followed by its characterization in terms of composition, structure, and mechanical properties that confirms the preservation of important characteristics of the arterial ECM. This unique hydrogel was then tailored into an angiogenesis model comprising endothelial and supporting cells, in a configuration that allowed high throughput quantitative analysis of cell viability and proliferation, cell migration, and apoptosis, revealing the advantages of paECM over frequently used biomaterials. Particularly, when applied with well-known angiogenesis effectors, the model enabled to measure and reflect the expected response of the effectors. Hence, validating its efficacy and establishing its potential as a promising tool for angiogenesis research.

Keywords

Arterial ECM; Angiogenesis; hydrogel

Abstract **#1787**

ENGINEERING PORCINE EXTRACELLULAR MATRIX HYDROGELS AS A TISSUE-SPECIFIC BIOACTIVE MICROENVIRONMENT FOR REGENERATIVE MEDICINE

Tzila Davidov[1], Marcelle Machluf[1]

[1] *Technion - Biotechnology and Food Engineering*

Corresponding author's email: tzila937@campus.technion.ac.il

Injectable cell delivery systems for regenerative therapy offer a minimally invasive administration while shielding and retaining the transplanted cells. Furthermore, the use of biomimetic delivery systems can increase cell survival and re-acclimating in the tissue. Due to its unique bioactivity, porcine extracellular matrix (pECM) —the natural bed of cells in the tissues—holds a great promise as a biomaterial for cell delivery. Our aim was, therefore, to develop tissue-specific pECM-based injectable systems for cell delivery. ECM, isolated from different porcine tissues was enzymatically processed into thermally induced pECM hydrogels, which were further assessed to investigate their distinctive properties, and their consequent ability to support cells for biomedical applications. In terms of composition, each hydrogel had preserved the main collagens and GAGs of its original tissue. Nevertheless, the collagenous profile varied between the different pECM hydrogels leading to microstructural differences in porosity and fiber size distribution. The rheological properties of the hydrogels were also evaluated, revealing soft gel behavior that varied in strength according to the pECM tissue of origin. These hydrogel characteristics highly affected their interactions with cells demonstrated through the different morphology and viability of mesenchymal stem cells (MSCs). Most importantly, the different hydrogels guided a tissue-specific spontaneous differentiation of human-induced pluripotent stem cells (hiPSCs), demonstrated through immunostaining and CEL-Seq analyses for tissue-specific gene expression.

To conclude, pECM-based cell delivery systems benefit unique, tissue-specific attributes that affect their properties and interactions with resident cells and generate a natural supportive bed for improved cell function and in vivo transplantation outcomes.

Keywords

hydrogel; hiPSCs; Tissue-specific

Abstract **#1788**

PHOTOTUNABLE INTERPENETRATING POLYMER NETWORK HYDROGELS TO STIMULATE THE VASCULOGENESIS OF STEM CELL-DERIVED ENDOTHELIAL PROGENITORS

Cody Crosby[P1,2], Alex Hillsley[2], Sachin Kumar[2], Brett Stern[2], Sapun Parekh[2], Adrianne Rosales[2], Janet Zoldan[2]

[1] *Southwestern University*

[2] *The University of Texas at Austin*

Corresponding author's email: crosbyc@southwestern.edu

Vascularization of engineered scaffolds remains a critical obstacle hindering tissue engineering translation from the bench to the clinic. We previously demonstrated the robust micro-vascularization of collagen hydrogels with induced pluripotent stem cell (iPSC)-derived endothelial progenitors; however, physically cross-linked collagen hydrogels compact rapidly and exhibit limited strength [1]. We have synthesized an interpenetrating polymer network (IPN) hydrogel comprised of collagen and norbornene-modified hyaluronic acid (NorHA) to address these challenges [2]. This dual-network hydrogel combines the natural cues presented by collagen's binding sites and extracellular matrix (ECM)-mimicking fibrous architecture with the in situ modularity and chemical cross-linking of NorHA. We modulated the IPN hydrogel's stiffness and degradability by varying the concentration and sequence, respectively, of the NorHA peptide cross-linker. Rheological characterization of the photo-mediated gelation process revealed that the IPN hydrogel's stiffness increased with cross-linker concentration and was decoupled from the bulk NorHA content. Conversely, the swelling of the IPN hydrogel decreased linearly with increasing cross-linker concentration. Collagen microarchitecture remained relatively unchanged across cross-linking conditions, although the addition of NorHA delayed collagen fibrillogenesis. Upon iPSC-derived endothelial progenitor encapsulation, robust, lumenized microvascular networks developed in IPN hydrogels over two weeks. Subsequent computational analysis showed that an initial rise in stiffness increased the number of branch points and vessels, but vascular growth was suppressed in high stiffness IPN hydrogels. These results suggest that an IPN hydrogel consisting of collagen and NorHA is highly tunable, compaction resistant, and capable of supporting vasculogenesis.

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Abstract **#1789**

BONE REGENERATION IN RAT CALVARIA DEFECT BOOSTED THROUGH A BIOMIMETIC COATED COLLAGEN MEMBRANE

Marie DUBUS$_P$[1,2], Loïc Scomazzon[1], Adrien Baldit[3], Abdelilah Beljebbar[4], Hassan Rammal[1], Fany Reffuveille[1,5], Julien Braux[1,2,6], Cédric Mauprivez[1,2,6], Halima Kerdjoudj[1,2]

[1] *Université de Reims Champagne Ardenne, Biomatériaux et Inflammation en Site Osseux (BIOS) EA 4691, Reims, France*

[2] *Université de Reims Champagne Ardenne, UFR d'Odontologie, Reims, France*

[3] *LEM3-UMR-7239, CNRS - Arts et Métiers ParisTech; ENIM, Université de Lorraine, Metz, France*

[4] *BioSpectroscopie Translationnelle (BioSpecT), EA 7506, SFR CAP-Sante FED4231, Université de Reims Champagne-Ardenne, UFR de Pharmacie, Reims, France*

[5] *Université de Reims Champagne Ardenne, UFR de Pharmacie, Reims, France*

[6] *Pôle Médecine bucco-dentaire, Hôpital Maison Blanche, Centre Hospitalier Universitaire de Reims, France*

Corresponding author's email: marie.dubus@hotmail.com

Guided bone regeneration (GBR) is an attractive technique for restoring oral bone defects, where an occlusive membrane provides space maintenance required to seclude soft tissue infiltration. However, bone regeneration can be impeded by a lack of an adequate tissue vascularization and/or by bacterial contamination. A bone inspired coating made of calcium phosphate-chitosan-hyaluronic acid was built on one side of a GBR collagen membrane. The resulting hybrid coating is composed of amorphous calcium phosphate and carbonated poorly crystalline hydroxyapatite, wrapped within chitosan/hyaluronic acid complex. Hybrid coated membrane possesses excellent bioactivity and capability of inducing an overwhelmingly positive response of stromal cells and monocytes in favor of bone regeneration [1,2,3,4]. Furthermore, the hybrid coating disturbs the cell wall integrity of Gram-positive and Gram-negative bacteria. Its combination with stromal cells, able to release antibacterial agents and mediators of the innate immune response, constitutes an excellent strategy for fighting bacteria [5]. A preclinical in vivo study was therefore conducted in rat calvaria bone defect for 8 weeks. µCT reconstructions showed that hybrid coated membrane favored bone regeneration (two-fold increase in bone volume / total volume ratios vs. uncoated membrane). The histological characterizations revealed the presence of mineralized collagen (Masson's Trichrome and Von Kossa stain) which was not fully organized (second harmonic analysis), but immunohistochemistry analysis highlighted a bone vascularization. Finally, Raman spectroscopy and biomechanical characterizations are in progress. This bioinspired hybrid coating provides a suitable environment for bone regeneration and vascularization, as well as an ideal strategy to prevent bone implant-associated infections.

Keywords

Biomimetic hybrid coating; Collagen membrane; Rat calvaria model

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Abstract **#1790**

INTERACTION BETWEEN CUTIBACTERIUM ACNES AND HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS: A STEP TOWARD UNDERSTANDING BONE INFECTION

Marie DUBUS$_P$[1,2], Jennifer Varin-Simon[1], Steve Papa[1], Cédrice Mauprivez[1,2], Céline Mongaret[1,3], Grégory Francius[4], Fabienne Quilès[4], Sophie. C. Gangloff[1,3], Fany Reffuveille[1,3], Halima Kerdjoudj[1,2]

[1] *Université de Reims Champagne Ardenne, Biomatériaux et Inflammation en Site Osseux (BIOS) EA 4691, Reims, France*

[2] *Université de Reims Champagne Ardenne, UFR d'Odontologie, Reims, France*

[3] *Université de Reims Champagne Ardenne, UFR de Pharmacie, Reims, France*

[4] *CNRS, UMR 7564, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement (LCPME), Université de Lorraine, Nancy, France*

Corresponding author's email: marie.dubus@hotmail.com

Upon reaching an injured site, mesenchymal stem cells (MSCs) are known to regulate the innate immune response of host and exerting antibacterial effects. However, few studies reported that bacteria as commensal Cutibacterium acnes (C. acnes) hidden from the immune response, inside MSCs, could cause implant-associated infection1,2. The present study hypothesizes that bone marrow could be contaminated during bone surgery with C. acnes and MSCs might serve as a protective niche for C. acnes.

Human bone marrow MSCs were challenged with two clinical and one laboratory C. acnes strains at MOI 30:13. Whatever their origin, C. acnes were able to invade MSCs, inducing the potential transition of commensal C. acnes to an opportunistic pathogen (i.e. by increasing biofilm formation and resistance of macrophage phagocytosis). Although direct and indirect antibacterial effects of MSCs secretome were not enhanced following C. acnes infection, C. acnes strains affect MSCs paracrine activities by promoting the secretion of immunomodulatory mediators and pro-healing growth factors: following 48h post-infection, a significant increase in IL-6, IL-8, PGE-2, VEGF, TGF-β and HGF release in infected MSCs supernatant was observed. IL-1$\beta$and TNF-α were not detected in all studied conditions. Moreover, an increase in OPG production suggested that MSCs/C. acnes interaction hinders bone homeostasis.

These results showed a direct impact of C. acnes on bone marrow MSCs, providing new insights into the development of C. acnes during implant-associated infections. Additional experiments using osteoblasts or osteoclasts co-cultures are required to state about the effect of C. acnes infection on bone healing and remodelling.

Keywords

C. acnes; Mesenchymal stem cells; Bone-associated infections

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Abstract **#1791**

NOVEL 3D PRINTING STRATEGY TO DESIGN ANISOTROPIC AND MACROPOROUS DENSE COLLAGEN HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS

Marie Camman[P][1,2], Onnik Agbulut[2], Christophe Hélary[1]

[1] Sorbonne Université, CNRS, UMR 7574, Laboratoire de Chimie de la Matière Condensée de Paris, F-75005 Paris, France

[2] Sorbonne Université, Institut de Biologie Paris-Seine (IBPS), CNRS UMR 8256, Inserm ERL U1164, Biological Adaptation and Ageing, 7 quai St-Bernard (case 256), F-75005, Paris, France.

Corresponding author's email: marie.camman@sorbonne-universite.fr

Collagen hydrogels, fabricated from low concentrated collagen solutions, are characterized by poor mechanical properties and low physical stability1. To circumvent these drawbacks, high collagen concentrations can be used but this increases cell mortality due to poor diffusion of nutrients and oxygen2. Moreover, cells such as cardiomyocytes or neurons only grow and organize themselves on anisotropic substrates3,4. The aim of this study was to develop a novel 3D printing technique using dense collagen solutions to design macroporous and anisotropic hydrogels. By modification of the extrusion velocity and the gelling method, diameter of aligned fibrils and hydrogel mechanical properties could be tuned. The phosphate buffer enabled anisotropy occurrence as observed by polarized light and SHG microscopy. To increase mechanical properties, the phosphate buffer gelation was combined with ammonia vapors to reach Young's moduli of physiological extracellular matrices (10 kPa). Fibroblasts seeded on top of anisotropic hydrogels aligned along the axis of 3D printed constructs. In addition, the process enabled to generate an intrinsic porosity of 20-40 µm in diameter between the different layers. This porosity is an asset for vascularization or neuronal guidance. To increase the scaffold porosity, the addition of macroporous channels with sacrificial matrix printing or needles molding created larger channels ranging from 100 to 500 µm. These bigger channels were adequate for the 3D organotypic organization of cardiomyocytes. Taken together, these results demonstrate the usefulness of 3D printing of dense collagen solutions to develop anisotropic and porous biomimetic scaffolds for tissue engineering applications.

Keywords

3D printing; anisotropy; porosity

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Abstract **#1792**

BIOFABRICATION OF INNERVATED TISSUE ENGINEERED MUSCLE (INTEM) FOR NEUROMUSCULAR REGENERATION FOLLOWING VOLUMETRIC MUSCLE LOSS

Suradip Das[P][1,2], Kevin Browne[1,2], Franco Laimo[1,2], James Lim[1,2], Joseph Maggiore[1], Melanie Hilman[1,2], Zarina Ali[1], Foteini Mourkioti[1], Wenli Yang[1], H. Isaac Chen[1,2], D. Kacy Cullen[1,2]

[1] *University of Pennsylvania*

[2] *Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia*

Corresponding author's email: suradip@pennmedicine.upenn.edu

Volumetric muscle loss (VML) is the traumatic or surgical loss of skeletal muscle beyond the inherent regenerative capacity of the body, generally leading to severe functional deficit. Formation of appropriate motor innervations remain one of the biggest challenges for both autologous grafts as well as tissue-engineered muscle constructs. We have recently fabricated Innervated Tissue Engineered Muscle (InTEM) comprising networks of spinal motor neurons and skeletal myocytes on aligned nanofibrous sheets. Pre-innervation resulted in formation of neuromuscular junctions in vitro as well as promoted myocyte fusion. Subsequently, the InTEMs were implanted in a rodent VML model and exhibited structural integrity and cell survival up to three weeks post repair. InTEMs significantly increased satellite cell density, neuromuscular junction maintenance, graft revascularization, and muscle volume over three weeks as compared to myocyte-only constructs and nanofiber scaffolds alone. These pro-regenerative effects can potentially enhance neuromuscular regeneration following VML, thereby improving the levels of functional recovery following these devastating injuries. Considering that bulk restoration is a major challenge following VML, we are working towards scaling up the InTEMs to fabricate thicker constructs by stacking multiple layers of cell laden nanofiber sheets. Further, we demonstrate fabrication of InTEMs using primary human skeletal myocytes and iPSC derived motor neurons to augment potential clinical translation of InTEMs using patient specific cell populations. These human InTEMs are being studied in a clinically relevant delayed repair model of VML so as to provide trauma surgeons, a patient specific solution to repair VML grade musculoskeletal injuries and facilitate functional restoration.

Keywords

volumetric muscle loss; innervation; aligned nanofiber

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Abstract **#1793**

PRINTABILITY AND CYTOCOMPATIBILITY OF A PHOTO-INITIATOR-FREE BIOINK BASED ON COUMARIN-MODIFIED HYALURONIC ACID AND GELATIN

Matteo D'Este[1], Riccardo Beninatto[2], Alba Di Lucia[2], Flurina Staubli[1], Andrea Schwab[1], Devis Galesso[2], Mauro Pavan[2]

[1] *AO Research Institute Davos*

[2] *Fidia Farmaceutici S.p.A.*

Corresponding author's email: matteo.deste@aofoundation.org

Photocrosslinking enables precise control over shape fixation in extrusion-based 3D bioprinting. However, most photocrosslinking processes entail propagation of free radicals with potential toxicity or longer-term impact on cell function. The aim of this study was to introduce a bioink undergoing a rapid photoinitiator-free photocrosslinking process without propagation of free radicals. The system is based on the coumarin derivatives of hyaluronic acid (HA) and gelatin (Gel), undergoing photodimerization.

Coumarin-modified HA and Gel characterized via NMR were used to prepare a range of formulations for an optimization parametric study; crosslinked HA was added as viscoelastic enhancer. Rheological properties (viscosity curve, elastic recovery, gelation time) were assessed using the AntonPaar Rheometer. Printability was assessed (3D Discovery, RegenHU) evaluating filament formation, uniformity and spreading, and the ability of the printed filament to support itself when printed over pillars (2-8 mm). Cytocompatibility was tested according to ISO 10993-5:2012 with Alamar blue on Balb/3T3 cell line.

The dimerization mechanism of coumarin moieties upon near-UV irradiation (365 nm) via photocycloaddition reaction did not form any by-products. Shear thinning behavior and instantaneous elastic recovery has been proven for several formulations. Upon extrusion, a uniform filament with little spreading was achieved, thus indicating high shape fidelity. Cell viability ranged between 77% (undiluted samples) and 100%. High cell viability was shown after encapsulated in different formulations. Differentiation of human mesenchymal stromal cells in these bioinks towards different lineages is currently under investigation.

The photo-initiator free bioink platform here introduced expands the possibilities of using biofabrication approaches for soft tissue engineering.

Keywords

Hyaluronic Acid; Biofabrication; Gelatin

Abstract **#1794**

A NOVEL DECELLULARIZATION METHOD OF ASCENDING THORACIC AORTA: A SCAFFOLD TOWARDS IN VITRO STUDY OF THE EXTRACELLULAR MATRIX ROLE IN ANEURISM PROGRESSION

Rosaria Santoro[P][1], Gianluca Lorenzo Perrucci[1], Federica Potere[2], Andrea Giorgia Bonora[1], Federica Boschetti[2,3], Giulio Pompilio[1,4]

[1] *Unità di Biologia Vascolare e Medicina Rigenerativa, Centro Cardiologico Monzino IRCCS, Milano, Italy*

[2] *Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta", Politecnico di Milano, 20133, Milano, Italy*

[3] *IRCCS Istituto Ortopedico Galeazzi, Milano, Italy*

[4] *Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università degli Studi, Milano, Italy*

Corresponding author's email: rsantoro@ccfm.it

Ascending thoracic aortic aneurysm (TAA) is a high incidence pathology, still lacking decryption of the underlying detrimental molecular mechanisms guided by cell-extracellular matrix (ECM) interaction [1]. In this work, we compared different decellularization methods of thoracic aorta (TA). TA samples obtained from healthy donors (n=4, cryopreserved specimen from tissue biobank) or patients affected by TAA undergone surgical aortic replacement (n=3) were treated using either i) snap freezing (24-48hours), ii) anionic detergent (sodium dodecyl sulfate, SDS, 48-72hours 1%w/v), iii) non-ionic detergent (24hours, Triton-X100, 0.1%w/v) followed by SDS (24hours, 0.1%w/v) [2] or iv) snap freezing (24hours) followed by SDS 1%w/v. Decellularization efficacy was evaluated in respect to cell removal and ECM integrity. At this aim, we performed nuclei (DRAQ5) and ECM component (vitronectin, collagen) immunofluorescence, histological analysis (haematoxylin & eosin, Masson's Trichrome) and immunohistochemistry (hydroxyproline). Results lead to identification of one protocol (48hours- SDS1%W/v) able to efficiently remove the cellular component, preserving ECM structure and geometrical organization. Finally, we verified that the selected protocol did not diminish tissue mechanical response, as demonstrated by the absence of statistically relevant differences between native and decellularized tissue in elastic moduli measured by tensile test in aorta radial direction (T-test: E1 p=0.64, E2 p=0.33). On the other side, decellularization procedure induced an increase in tissue permeability , thus supporting its exploitation as novel scaffold for in vitro study of the interaction between aorta resident cells and ECM, in the contest of TAA pathological mechanisms (e.g. mechanotransduction pathways).

Keywords

thoracic aortic aneurysm; decellularization; scaffold

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Abstract **#1796**

AN INNERVATED SYNOVIUM-CARTILAGE CHIP FOR MODELING JOINT INFLAMMATION AND ASSOCIATED PAIN

Zhong Li[1], Meagan J Makarcyzk[1], Jamie K Moy[1], Ilhan Yu[1], Fang-wei Liu[1], Qi Gao[2], Sung Kwon Cho[1], Douglas J Weber[1], Bruce A Bunnell[3], Stuart B Goodman[2], Michael S Gold[1], Hang Lin$_P$[1]

[1] *University of Pittsburgh*

[2] *Stanford University*

[3] *University of North Texas Health Science Center*

Corresponding author's email: hal46@pitt.edu

Joint diseases such as osteoarthritis are highly prevalent and are often accompanied with synovial inflammation (synovitis), cartilage degradation, and pain. Currently, neither effective disease-modifying drugs nor efficacious and safe medications for long-term pain management are available. To address this unmet medical need, we propose to develop an innervated, human cell-derived microphysiological system for understanding joint pain mechanisms and developing pain medications.Polydimethylsiloxane-based microfluidic chips were fabricated to culture three-dimensional synovium and cartilage tissues engineered from human mesenchymal stem cells (hMSC). Given their crucial role in inducing “synovitis”, macrophages derived from human peripheral blood monocytes were co-encapsulated in the synovial tissue with hMSCs-derived fibroblasts. The real-time synovium-cartilage crosstalk was enabled by a “simulated synovial fluid (SSF)” shared by these two tissues. A separate neuron chamber that housed human or rodent dorsal root ganglion neurons was connected to the synovium chamber via microchannels, through which the neurites extend to innervate the synovium. Of note, neurons were indirectly influenced by cartilage and SSF through synovial tissues. “Synovitis” was induced by polarizing the macrophages toward the proinflammatory M1 phenotype or challenging synovium with the pro-inflammatory cytokine interleukin-1β. The inflamed synovium caused cartilage degradation and the generation of pain mediators, including Prostaglandin E2, tumor necrosis factor-α, and C-telopeptide of type II collagen, in the SSF. Furthermore, the inflamed synovium evoked an increase in calcium efflux in a subpopulation of neurons.These results indicate that this novel innervated, human cell-derived system can be potentially employed for understanding inflammation-mediated joint pain and developing effective pharmaceutic interventions.

Abstract **#1797**

PSYCHOSOCIAL AND ETHICAL FACTORS AFFECTING PATIENTS' DECISION MAKING ABOUT UPPER EXTREMITY VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Elisa J Gordon[P][1], Brianna Kuramitsu[1], Jessica Gacki-Smith[1], Alexander Ferzola[2], Karen Vanterpool[2], Christina Kunkle[3], Melissa Hewitt[3], Andrea Schultheis[3], Tiffany Riggleman[3], Jerika Taylor[3], Carisa Cooney[2], Scott Tintle[3], Gerald Brandacher[2], Macey Levan[2]

[1] *Northwestern University Feinberg School of Medicine*

[2] *Johns Hopkins University*

[3] *Walter Reed National Military Medical Center*

Corresponding author's email: e-gordon@northwestern.edu

The informed consent process for upper extremity (UE) Vascularized Composite Allotransplantation (VCA) has not been standardized. Information provided to patients varies, which may contribute to individuals being inadequately informed and under-prepared for decision making. Therefore, this study examined psychosocial factors affecting decision-making about UE VCA. We conducted in-depth interviews among people with acquired UE amputations. Open-ended questions assessed psychosocial factors informing decision making about UE VCA. Thematic analysis was used to analyze qualitative data. To date, 18 people completed in-depth interviews (82% participation rate), including 3 undergoing UE VCA evaluation. Most were male (78%), had a mean age of 48 years, had a unilateral amputation (78%), and had undergone amputation a mean of 10 years earlier. Fifty percent were 'completely' or 'a lot' willing to pursue VCA. Psychosocial factors influencing decisions to pursue VCA included: regaining functionality and the associated independence; increasing social and physical confidence; and enabling more active parental involvement in childrearing. Psychosocial factors and concerns influencing decisions not to pursue VCA included: health or limb function becoming "worse off" from UE VCA; the rigorous commitment required for undergoing UE VCA and rehabilitating; and having already adapted to life without upper limb(s). Participants would ethically justify getting an UE VCA if the UE VCA success rate was high and risks were low, and if participants currently struggled with managing daily tasks. Preliminary findings suggest that people with UE amputations hold concerns that diminish enthusiasm for UE VCA. Addressing psychosocial concerns may foster informed decision making about UE VCA.

Abstract **#1798**

SIMPLE STRATEGIES TO DEVELOP 3D BREAST CANCER TUMOR MICROENVIRONMENT WITH M2 MACROPHAGE VIA INDUCED GM-CSF AND G-CSF EXPRESSION

Na Ly Tran[1,2], In Kyu Lee[2], Sang-Heon Kim[2,1], Seung Ja Oh[2]

[1] *Division of Bio-Medical Science & Technology, Korea University of Science and Technology, Daejeon 305-350, Korea.*

[2] *Biomaterial Research Center, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu,Seoul, 02792, Republic of Korea.*

Corresponding author's email: 618004@kist.re.kr

The macrophage is a type of phagocyte that plays a critical role in the immune system. It is well known that macrophage is polarized to tumor-associated macrophage (TAM) and promotes cancer progression and drug resistance in the tumor microenvironment(TME). Although this increasing knowledge on macrophage in the TME, there are technical hurdles to develop in vitro models to simulate immunocompetent TME. Here, we propose simple strategies to develop the 3D breast cancer tumor microenvironment with M2 macrophages via induced GM-CSF and G-CSF expression. To develop in vitro immunocompetent TME for breast cancers, we first formed the 3D multicellular cancer spheroids containing invasive human breast cancer (MDA-MB-231) and THP-1 driven M0 macrophages. Next, we analyzed the 3D multicellular cancer spheroids and found that the 3D architecture of cancer cells induces M2 polarization of macrophages in in vitro immunocompetent TME for breast cancer. Then, we demonstrated enhanced expression of GM-CSF and G-CSF via 3D breast cancer spheroids increases transcript activation of pSTAT-3, pp38, pNf-kB, thereby inducing M2-like macrophage polarization. Finally, we confirmed the enhanced drug resistance by M2-like macrophage polarization in in vitro 3D immunocompetent cancer model. The simple strategy to form in vitro 3D immunocompetent TME developed in this study will contribute to many biological fields constating academies and industries for precise evaluation of cancers.

Keywords

3D immunocompetent cancer model; the tumor microenvironment; Tumor-associated macrophage (TAM)

Abstract **#1799**

THE EFFECT OF SUBSTRATE TOPOGRAPHY AND GEOMETRY ON THE PHENOTYPIC EXPRESSION OF MICROGLIA

Angelo Accardo[1], Ahmed Sharaf$_P$[1], Brian Roos[1], Raissa Timmerman[2], Jeffrey Bajramovic[2]

[1] *Delft University of Technology, Delft, The Netherlands*

[2] *Biomedical Primate Research Centre, Rijswijk, The Netherlands*

Corresponding author's email: A.M.S.E.Sharaf@tudelft.nl

When we talk about cell culture, we can discriminate between 2D and 3D models. In 2D models, biologists culture cells onto flat glass slides and, although this approach is easy to handle and cheap, it often leads to the formation of unrealistic cell monolayers which do not reproduce the complex 3D spatial configuration that we find in the brain. For this reason, during the last two decades, novel strategies started to emerge and led to the creation of biomimetic 2.5D and 3D cell microenvironments [1]. Among the available fabrication techniques, two photon direct laser writing [2,3] allows the creation of complex architectures by photopolymerizing extremely confined volumes (voxels) via focusing femtosecond laser pulses onto an organic photosensitive pre-polymer material. In this work we report the development of 2.5D and 3D constructs to unveil the behavior of primary microglia cells extracted from primate models. Within ex-vivo models, microglia preserve their natural ramified “resting” state but as soon as they are cultured in a 2D environment, they often assume an “activated” amoeboid configuration. Here we show how artificial structures can promote a higher ramified configuration in vitro

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Abstract **#1800**

PLASMA-TREATED FLEXIBLE MULTISCALE NANOTOPOGRAPHIC PATCHES FOR SOFT AND HARD TISSUE ENGINEERING

Woochan Kim[P][1], Yonghyun Gwon[1], Sunho Park[1], Jangho Kim[1]

[1] *Chonnam National University*

Corresponding author's email: rladncks92@gmail.com

The design of functional scaffolds as transplantable platforms for tissue repair and regeneration requires gaining precise control of surface properties. Here, we propose a new methodology to fabricate hierarchical multiscale nanotopographic scaffolds with controlled hydrophilic properties by employing capillary force lithography in combination with plasma modification. Using our method, we developed biodegradable and biocompatible polymer (i.e., polycaprolactone (PCL))-based multiscale nanotopographic scaffolds with natural extracellular matrix-like hierarchical structures along with flexible and controlled hydrophilic properties by treating nitrogen and oxygen gas plasma. In response to multiscale nanotopography and chemically modified surface, the proliferation and osteogenic mineralization of cells were significantly promoted. Furthermore, the multiscale nanotopographic scaffolds promoted regeneration of the rotator cuff tendon tissue and the calvarial bone tissue in vivo in rat models. Overall, the PCL-based multiscale nanotopographic scaffolds could accelerate soft and hard tissue regeneration. Thus, our proposed methodology was confirmed as an efficient approach for the design and manipulation of scaffolds having a multiscale nanotopography with controlled polymeric surface property.

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Abstract **#1801**

ESTIMATION OF CANCER INVASION THROUGH EXTRACELLULAR MATRIX REMODELING ON PHASE-ENGINEERED GRAPHENE OXIDE

Chien-YU Huang[P][1,2]

[1] *Department of Electrical and Computer Engineering, College of Electrical and Computer Engineering, National Yang Ming Chiao Tung University, Hsinchu, Taiwan*

[2] *Institute of Biomedical Engineering, National Yang Ming Chiao Tung University, Hsinchu, Taiwan*

Corresponding author's email: apple21038526@gmail.com

Cancer metastasis is the major cause of death for malignant tumor nowadays. In the process of cancer metastasis, cancer-associated fibroblasts (CAFs) play a key role in the tumor microenvironment, inclusive of extracellular matrix (ECM) deposition, angiogenesis, tumor proliferation as well as metastasis, and chemoresistance. Nevertheless, many distinct features and interaction between CAFs and cancer cells in the tumor microenvironment, termed the niche, remains unclear. To investigate the mechanism of how CAFs regulate cancer invasion, as well as the estimation of invasion behavior a variety of cancer cells, this research aims to establish a complex biomimetic tumor microenvironment via a nanomaterial based interface with CAFs. In this study, we induced a phase transformation in graphene oxide (GO), leading to different degrees of cluster of oxygen groups and dissimilar efficiency of growth factors conjugation. We found that the GO interface results in ECM deposition, such as collagens, from CAFs, which can be enhanced when the interface is conjugated with platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor-β (TGFβ). Furthermore, the nanomaterial based interface with CAFs affect the motility features of cancer cells, including migration rate and orientation. Apart from this, clinical samples will be next added into our work to further investigate the cell-cell interaction. Overall, we demonstrate the potential of CAFs with GO for cancer invasion mechanism research, which may possibly be applied in potential drug development targeting CAFs and achieve personalized precision medicine in the future.

Keywords

cancer-associated fibroblasts (CAFs); Extracellular matrix (ECM) remodeling; Graphene Oxide (GO)

Abstract **#1802**

APPLICATION OF ADVANCED BIOENGINEERED SKIN CONSTRUCTS FOR SKIN HEALTH RESEARCH

Lydia Costello$_P$[1], Kirsty Goncalves[1], Amy Simpson[1], Lucy Smith[1], Matthew Freer[1], Victoria Maltman[1], Paola De Los Santos Gomez[1], Pamela Ritchie[1], Ben Hulette[2], Ryan Tasseff[2], Teresa Dicolandrea[2], Kevin Mills[2], Robert Isfort[2], Charlie Bascom[2], Stefan Przyborski[1,3]

[1] *Department of Biosciences, Durham University, Durham, UK*

[2] *P&G Mason Business Centre, Cincinnati, Ohio, US*

[3] *Reprocell Europe, Sedgefield, UK*

Corresponding author's email: lydia.f.costello@durham.ac.uk

Tissue engineered skin constructs are valuable tools for academic and industrial research, to investigate skin physiology and disease, and provide a predictive platform for pre-clinical studies. We have developed advanced, complex skin equivalents that resemble the structure and function of human skin, and have multifaceted applications in skin health research.

To mimic the dermal composition in vitro, primary fibroblasts were incorporated within the porous, polystyrene Alvetex® Scaffold, to synthesise endogenous extracellular matrix proteins. The dermal compartment supports the formation of a differentiated, stratified and keratinised epidermis, with mature barrier properties. Neonatal, young and ageing cells have been used to bioengineer age-specific skin models. Additional cell types such as melanocytes and immune cells have also been successfully incorporated to generate complex skin constructs.

Bioengineered skin equivalents recapitulate the microanatomy of human skin in vitro. Ageing skin equivalents demonstrate an age-related phenotype, with epidermal thinning and reduced synthesis of extracellular matrix proteins.

Complex skin constructs with additional cell types have been generated, with correct strata-specific localisation and functionality. Uniformly pigmented skin constructs contain melanocytes localised to the basal layer, which successfully transfer melanin to neighbouring keratinocytes. These skin models exhibit a darker pigmentation in response to ultraviolet radiation. Immune-competent skin constructs contain Langerhans cells in the epidermis, which are responsive to allergens and irritants.

The advanced, complex skin constructs have been tailored for downstream academic and industrial applications, such as investigating the molecular mechanisms of skin ageing, photoprotection research and determining the efficacy of actives in skin care formulations.

Keywords

Organotypic model; Skin biology

Abstract **#1803**

IL-1B-PRIMED MESENCHYMAL STROMAL CELL POTENTIATE AUTOLOGOUS SKIN ENGRAFTMENT IN A SEVERE BURN RAT MODEL

Marina Trouillas[P][1,2], Marianne Dedier[1,2], Arnaud Baus[1,2,3], Muriel Nivet[1,2], Sébastien Banzet[1,2]

[1] *IRBA, French Armed-forces Biomedical Research Institute, Clamart, France*

[2] *INSERM U1197, Villejuif, France*

[3] *Plastic Surgery Department, Percy Hospital, Clamart, France*

Corresponding author's email: trouillasmarina@gmail.com

One of the determining factors for the survival of severe burn patient is the rapid wound coverage and definitive closure of burn lesions. Despite the great diversity of approaches, the prognosis for severe burns remains unsatisfactory today with poor skin engraftment that often results in a pathological scar. Since their discovery, Mesenchymal Stromal Cells (MSCs) have aroused growing therapeutic interest for skin repair, thanks to their trophic, their immunomodulatory, their cell matrix remodeling, and their pro-angiogenic properties. The discovery of their plasticity with regard to various environmental stimuli has more recently opened up new therapeutic perspectives, such as priming strategies. Recently, our laboratory showed that IL-1β priming of MSC improves their wound healing potential in a model of skin excision.
In this study, we investigated whether IL-1β-primed MSC could improve autologous skin graft take in a severe burn rat model. Syngeneic IL-1β-primed MSC, derived from bone marrow, were evaluated in a 3rd degree rat burn model. The development of this model was inspired on surgical management of severe burn patient. This treatment consisted in a rapid excision of burned tissue and autologous split-thickness skin graft application to ratio 1:6 concomitants with the subcutaneous injection of MSC. Histology and immunohistochemistry analysis were used to monitor skin graft take, re-epithelialization, epidermis and dermis remodeling, angiogenesis and inflammation. We hope this animal model will help find an innovative MSC based-therapy for severe burn patients.

Keywords

Burn; Mesenchymal stromal cells; Priming

Abstract **#1804**

SUSTAINED RELEASE OF EXTRACELLULAR VESICLES USING ELECTROSPUN SCAFFOLD AS NOVEL APPROACH FOR CELL-FREE TISSUE ENGINEERING

Hatim Alqurashi[1,2], Stuart Hunt[2], Ilida Ortega Asencio2 Ortega Asencio[2], Daniel Lambert[2]

[1] *College of Dentistry, King Faisal University, Saudi Arabia*

[2] *2School of Clinical Dentistry, University of Sheffield, Sheffield, UK*

Corresponding author's email: halqurashi2@sheffield.ac.uk

Introduction: Extracellular vesicles (EVs) are membrane-enclosed vesicles that are secreted by cells and mediate cell–cell communication. EVs may provide opportunities for new tissue engineering (TE) approaches to mimic both the microscopic and molecular in vivo environment and to enhance tissue repair.

Methods: EVs were isolated from the conditioned media of normal oral fibroblast cells in culture. After characterising the size and protein abundance of EVs, they were incorporated into polycaprolactone electrospun scaffolds using conventional adsorption and covalent-attachment based methods. The number of EVs attached to the scaffolds and their release kinetics was examined using nanoparticle-tracking analysis (NTA). The influence of myofibroblast-derived EVs on cell migration was studied using scratch assays.

Results: EVs were successfully isolated by UC and SEC as assessed by NTA and determination of presence of EV marker proteins (CD9, CD63 and CD81). Our functionalisation comparative study indicated that the use of conventional adsorption (achieved after PCL activation) may offer a novel and simple approach for the homogeneous incorporation of EVs. For these cases, NTA showed slow release of 40% of EVs from the scaffolds over 21 days. Myofibroblast-derived EVs significantly increased fibroblast activation and migration compared to untreated controls.

Conclusion: Here we provide evidence that electrospun scaffolds can be functionalised with EVs and provide sustained slow release, offering an opportunity to develop novel, cell-free and tuneable approaches to tissue engineering. We also showed that myo-EV may have a role in wound healing, and that incorporation of myo-EVs in scaffolds may have potential as a regenerative medicine approach.

Abstract **#1805**

HIGH MOBILITY GROUP BOX 1 PROMOTES BONE REGENERATION DURING DISTRACTION OSTEOGENESIS VIA ACCELERATING ANGIOGENESIS

Qi Chang[P][1], Masahito Fujio[1], Makoto Tsuboi[1], Huiting Bian[1], Hideharu Hibi[1]

[1] *Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*

Corresponding author's email: tomeray@163.com

Purpose: This study aims to clarify the effect of high mobility group box 1 (HMGB1) in promoting healing in a mouse distraction osteogenesis (DO) model.

Methods: The effects of HMGB1 on the proliferative and migratory ability of human bone marrow mesenchymal stem cells (hBMSC) were evaluated in vitro. Osteogenic differentiation capacity was evaluated by alkaline phosphatase (ALP) and Alizarin Red S staining. Moreover, DO was performed on the tibia of the mice. The experiments were divided into three groups: HMGB1 with scaffold transplantation group (HMGB1 group), phosphate-buffered saline (PBS) with scaffold transplantation group (control group), and no transplantation group. New bone and callus formation were evaluated by microCT and hematoxylin and eosin staining. The angiogenesis was evaluated by immunofluorescence on days 10 and 20.

Results: HMGB1 enhanced the migration, mineralization ability, and ALP activity of hBMSC in vitro. HMGB1 group showed more bone formation than the other two groups. In addition, HMGB1 group has been shown to promote angiogenesis in the DO gap. Furthermore, the local HMGB1 application promotes bone healing by accelerating angiogenesis during the DO healing process.

Conclusion: HMGB1 is suggested to have a regenerative activity according to the acceleration of angiogenesis to shorten the healing period during DO.

Abstract **#1806**

LIPOFECTION OF CHEMICALLY MODIFIED MRNA IN IN VITRO CELL CULTURES

Claire Polain[P][1], Claudia Del Toro Runzer[1], Christian Plank[2], Elise Bonvin[3], Florent Poulhes[3], Martijn van Griensven[1,4], Elizabeth Rosado Balmayor[5,4]

[1] *CBITE Department, MERLN Institute, Maastricht University, Maastricht, The Netherlands*

[2] *Ethris GmbH, Planegg, Germany*

[3] *OZ Biosciences SAS, Marseille, France*

[4] *Rehabilitation Medicine Research Center, Mayo Clinic, Rochester, MN, USA*

[5] *IBE Department, MERLN Institute, Maastricht University, Maastricht, The Netherlands*

Corresponding author's email: c.polain@maastrichtuniversity.nl

Tissue regeneration often combine growth factors, cells and biomaterials. Gene therapy is considered to overcome growth factor limitations. pDNA, carried by viral vectors is traditionally used in gene therapy. This often results in risk for mutagenesis. Alternatively, mRNA has been proposed. Chemical modifications to mRNA (cmRNA) can increase its stability and biocompatibility. In order to transfer mRNA to cells, lipid vectors are commonly used. In this study, we evaluated the performance of diverse lipids in delivering cmRNA in vitro.

Lipoplexes were produced by using cmRNAs coding for Metridia Luciferase (MetLuc) and eGFP that were mixed with 6 different lipids. Ratios of cmRNA:Lipid tested were in the range of 0.5:1 to 1:11 (w/v). Doses of cmRNA starting from 0.78 pg/cell up to 50 pg/cell were investigated. For transfection, A549 and HEK293 cells were used. MetLuc expression was assessed by coelenterazine assay at 24-, 48-, and 72-hours post-transfection. PrestoBlue was performed to evaluate cell viability after transfection. Optimal cmRNA concentration at the best performing cmRNA:Lipid ratios were selected to transfect hMSCs. In hMSCs, MetLuc and eGFP expression was evaluated up to 7 days post-transfection. eGFP was assessed by flow cytometry and fluorescent microscopy.

Overall, concentrations of cmRNA >25 pg/cell in ratios >1:5 (cmRNA:Lipid) lead to increased cytotoxicity and decreased functionality. Using 6.25 pg cmRNA per cell in a 1:4 ratio with NL37 and 3D Fect showed to be the most efficient and biocompatible transfection condition. This was confirmed in hMSCs with prolonged expression up to 5 days.

Abstract **#1807**

AIR PULSE STIMULATION DEVICE DEVELOPMENT FOR 3D BIO PRINTING RECONSTRUCTED TISSUE

Hugo Marchal-Chaud$_P$[1]

[1] *VetAgro Sup_ Ecole vétérinaire de Marcy l'Etoile (FRANCE)*

Corresponding author's email: hugo.marchalchaud@gmail.com

Several mechanical stimulations have been used to improve the properties of tissue engineered constructs (TEC). The objective of the study was to develop a contactless stimulation process applied during the maturation of a 3D bioprinted dermal tissue. Our hypothesis was that air pulses would induce enough mecanotransduction to modify the mechanical, morphological and structural TEC properties. A dedicated air pulse stimulation device (APS) was developed and validated. The APS delivered an impinging jet with controlled pressure, airflow, speed and impinging height to transmitted waves through the object. The APS was designed by CAD and the 3D printing was manufactured in Acrylonitrile butadiene styrene to be adaptable to every culture plate with a switchable diameter of stimulation. The APC was also designed to ensure the tissue sterility during the stimulation. Range stimulation parameters were determined based on the literature and experimentally by testing the impact on acellular 3D bio-printed construct. Numerical simulation based on both the experimental outlet speed and the mechanical properties of the tissue measured by DMA (Dynamical mechanical Analysis) was perfomed.

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Abstract **#1808**

ACOUSTIC PROGRAMMING OF DEEP-ZONE CYTOARCHITECTURE IN TISSUE ENGINEERED CARTILAGE

James P K Armstrong[P][1], Sirli Treumuth[1], Ekaterina Pchelintseva[1], Bruce W Drinkwater[2], Molly M Stevens[1]

[1] Department of Materials, Department of Bioengineering, and Institute of Biomedical Engineering, Imperial College London, London, SW7 2AZ, United Kingdom

[2] Department of Mechanical Engineering, University of Bristol, Bristol, BS8 1TR, United Kingdom

Corresponding author's email: jamespaularmstrong@googlemail.com

Tissues frequently exhibit cellular organization that directs their development and function, and thus it is important to replicate these structural features when seeking to engineer tissue grafts or biological models [1]. We have previously shown that ultrasound standing waves can be used to rapidly and remotely form tunable arrays of viable cells for the engineering of aligned skeletal muscle [2]. Here, we have applied a similar methodology to generate 3D arrays of articular chondrocytes for the engineering of cartilage with deep-zone cytoarchitecture.

We fabricated acoustic cell patterning devices with pairs of piezotransducers to generate programmable ultrasound standing waves. We used these devices to pattern chondrocytes en masse into organized arrays predicted by pressure field modeling. The acoustic patterning process was rapid and reproducible, forming chondrocyte arrays with uniform separation (~111 µm) in under 5 s. Chondrocytes exposed for up to 10 min of ultrasound exhibited no measurable differences in membrane viability or metabolic activity, and retained their capacity for secreting cartilaginous extracellular matrix.

We next acoustically patterned chondrocytes throughout the 3D volume of agarose hydrogels, which we put through an established course of cartilage tissue engineering. Over the course of 35 days, the chondrocytes secreted large quantities of type II collagen and sulfated glycosaminoglycan while remaining in their acoustically-patterned configuration. To the best of our knowledge, this is the first example of an acoustically-patterned cartilage tissue and the first instance in which the deep-zone structure of cartilage has been engineered in vitro.

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Abstract **#1809**

A TOOL-PATH GENERATOR SOFTWARE FOR SCAFFOLDS BIOPRINTING

José Luis Dávila$_P$[1], Bruna Maria Manzini[1], Paulo Inforçatti Neto[1], Jorge Vicente Lopes da Silva[1]

[1] *Center for Information Technology "Renato Archer"*

Corresponding author's email: jose-luisd@hotmail.com

Tissue engineering is a promising area of regenerative medicine, which aims to repair, replace, and regenerate injured tissues and organs. For this purpose, scaffolds and biological constructs are fabricated to mimic the extracellular matrix of natural tissues. 3D bioprinting is a versatile technology to fabricate these structures. Nowadays, open-source 3D printer projects have made it possible to spread bioprinting at the laboratory scale. Hence, hardware and software developments are required to adapt the available projects to the bioprinting field. In this research, the software BioScaffolds PG V2.0 was developed to generate tool-paths for scaffold fabrication starting from parameterized geometries. The software was programmed using the VB.NET language and is compatible with the Fab@Home and RepRap variants open-source 3D printers. This tool allows obtaining tool-paths to fabricate scaffolds in the 3D printer platform, Petri dishes, and tissue culture plates. Moreover, it works independently from Computer-Aided Design (CAD) software. Consequently, the user can quickly define all the scaffold parameters to obtain a tool-path for bioprinting. The software was initially validated using CAM (Computer-Aided Manufacturing) simulations of the tool-paths. Next, tests were performed using a Fab@Home and a RepRap-based 3D printer with a piston-driven system. A shear-thinning ink was formulated for the validation stage. Finally, a hydrogel-based bioink was used to perform the bioprinting validation. After the validation process, it was concluded that the developed software is a powerful laboratory tool for bioprinting.

Keywords

Bioprinting; Scaffolds; Tool-path

Abstract **#1810**

INVESTIGATION OF REPARATIVE ACTIVITY OF THE NEW HYGIENIC PRODUCT FOR ORAL CAVITY

Olena Toporova$_P$[1], Liudmila Kravchenko[2], Olena Appelhans[2], Yana Ivanova[2]

[1] *Institute of Molecular Biology and Genetics NAS of Ukraine*

[2] *Odessa National Medical Univercity*

Corresponding author's email: sonshine22@ukr.net

Formula of an elixir for oral cavity on the basis of biologically active substances of bee products and natural compounds was developed, which requires detailed study. The study was carried out on a rounded aseptic plane wound.

Animals were divided into 4 groups: group 1 – a model of a plate wound with an untreated surface, group 2 (control) – the wound treated with sterile 0.9 % sodium chloride, group 3 (main) – the wound treated with the new elixir, group 4 (comparative) – the wound surface treated with comparator agent –"Phytopropolis".

Positive dynamics of wound healing was revealed visually in animals that received topical application of hygiene products based on propolis, which was confirmed by a quantitative calculation by the planimetry method.

The difference at the area of wound defect of the animals was determined on the 3rd day of the experiment. On the 7th day, it was determined that the wound area in the main group was significantly reduced as compared to the control and comparison groups. On the 14th day, the reduction of the wound area in the main group was significantly more pronounced as compared to other experimental animals and almost complete healing was observed. The difference in changes at the wound area in rats of the main and comparative group was on the 3rd day – 25.7 %, on the 7th day – 37.5% and on the 14th day – 45.2%.

Reparative activity of the new elixir was determined on the model of wound process.

Keywords

oral; healing; bee

Abstract **#1812**

«NOSE-TO-KNEE» TISSUE ENGINEERED AUTOLOGOUS NASAL CARTILAGE GRAFT TRANSPLANTATION FOR CHONDRAL LESIONS OF THE KNEE: 5-YEARS MID-TERM FOLLOW-UP

Marcus Mumme[1,2], Gyözö Lehoczky[1,2], Anke Wixmerten[2], Marcel Jakob[3], Andrea Barbero[2], Dorothee Harder[4], Sylvie Miot[2], Ivan Martin[2], Majoska Berkelaar$_P$[5]

[1] *Orthopedics and Traumatology, University Hospital Basel*

[2] *Department of Biomedicine, University of Basel*

[3] *Crossklinik Basel*

[4] *Radiology, University Hospital Basel*

[5] *University Hospital Basel*

Corresponding author's email: majoskahendrinamaria.berkelaar@usb.ch

Introduction: Engineered, autologous nasal cartilage grafts have been used recently in a first-in-human clinical trial (ClinicalTrials.gov: NCT01605201) Feasibility and safety were demonstrated, and the first promising clinical and radiological results were recorded up to two years postoperatively (1). We here report on clinical and radiological outcomes five years after implantation.Methods: 10 patients of phase I clinical trial were examined clinically and radiologically after a minimum of 5 years postoperative. Adverse events (AE) were collected. Clinical assessment was made using the KOOS questionnaire, and radiological results were evaluated using the MOCART scoring system. Results: Mean follow up was 63 months postoperatively. Mean age at implantation was 38.8 years, total size of treated cartilage lesions was in average 3.6 cm2. There were no serious adverse reactions 5 years postoperatively.Improvements of KOOS scores from preoperative (66.1) to 2 years (88.5) remained stable at 5 years (90.6). Subscales for Sports and QoL further improved from 2 years (78.00 and 68.13) to 5 years (84.50 and 79.38). Radiological analyzes showed improvements of joint effusion and bone marrow edema, but with overall slightly reduced MOCART scores from 58.8 (2 years) to 51.3 (5 years).Conclusion: This study confirms the safety and feasibility of treating focal cartilage lesions of the knee joint with tissue engineered, autologous nasal cartilage grafts at midterm. Patients benefit at 2 years is maintained over 5 years. Comparative studies with bigger cohort are required to better assess efficacy in comparison to standard therapies.

Keywords

Cartilage repair; Nasal chondrocytes; Clinical trial

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Abstract **#1813**

THERAPEUTIC EFFICACY OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS IN THE TREATMENT OF EXPERIMENTAL ISCHEMIC STROKE IN RATS

Olena Deryabina[P][1], Vasyl Moroz[2], Serhii Konovalov[2], Olena Toporova[1], Nadiia Shuvalova[1], Albert Tochylovskii[3], Vitalii Kordium[1]

[1] SI "Institute of Genetic and Regenerative Medicine" NAMS of Ukraine, Kyiv, Ukraine

[2] Vinnytsia National Medical University named after M. Pirogov, Vinnytsia, Ukraine;

[3] LLC "BioTexCom", Kyiv, Ukraine

Corresponding author's email: oderyabina@gmail.com

There are currently no sufficiently effective treatments for ischemic stroke, that leads to the development of innovative approaches such as cell therapy. The aim of the research was to study therapeutic effects of mesenchymal stem cells (MSCs) transplantation on stroke model in rats.

The studies were performed in male rats, which underwent a transient bilateral 20min. ischemia-reperfusion of internal carotid arteries. After modeling the pathology animals were injected with human UC-MSCs (1x106cells) into the femoral vein. Control animals received 0.2 ml of saline. The neurological status of the animals was assessed before ischemia induction and after cell transplantation on days 7 and 14.

Stem cell transplantation in experimental group promoted mortality reduction and animals life prolongation. At the 12th hour of observation (critical period in the development of experimental pathology) mortality in experimental group was 10% vs. 45% in control group ($p<0,05$). On the 7th day, the average score according to the Stroke-index McGrow scale was 7.14 ± 0.19 points in experimental animals vs 11.79 ± 0.48 in control group; on the 14th-4.86 ± 0.15 vs 9,14 ± 0.30($p <0.05$). MSC transplantation achieved active orientation restoration and research behavior indicators of animals. Cytofluorimetric study showed that experimental cell therapy reduced the intensity of nuclear DNA fragmentation in sensorimotor cortex neurons.

Thus, human umbilical cord MSC transplantation lowered mortality, alleviated neurological symptoms and normalized behavioral responses, ie, led to a significant regression of neurological deficits in rats with experimental ischemic stroke.

Keywords

ischemic stroke model ; UC-MSCs; cell therapy

Abstract **#1814**

BIOLOGICAL ASSESSMENT OF PROTEIN-BASED BIOMATERIALS AS POTENTIAL CANDIDATES FOR CORNEAL BIOENGINEERING

Cristina Romo-Valera[P][1], Pedro Guerrero[2,3], Jon Arluzea[1], Jaime Etxebarria[1,4], Koro de la Caba[2,3], Noelia Andollo[1,5]

[1] *Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country (UPV/ EHU), Barrio Sarriena S/N, 48940 Leioa, Spain*

[2] *BIOMAT Research Group, Chemical and Environmental Engineering Department, Engineering College of Gipuzkoa, University of the Basque Country (UPV/ EHU), Plaza de Europa 1, 20018 Donostia-San Sebastián, Spain*

[3] *BCMaterials, Basque Center for Materials, Applications and Nanostructures, UPV/EHU Science Park, 48940 Leioa, Spain*

[4] *Department of Ophthalmology, BioCruces Bizkaia Health Research Institute, University Hospital of Cruces, Begiker, Plaza de Cruces S/N, 48903 Barakaldo, Spain*

[5] *BioCruces Bizkaia Health Research Institute, Begiker, Plaza de Cruces S/N, 48903 Barakaldo, Spain*

Corresponding author's email: cristinaromo1@gmail.com

Corneal transplantation continues to be one of the leading treatments against vision impairments that affect million people worldwide. However, the shortage of donors shows the need of substitutes that mimic the native tissue to promote cell growth and the subsequent tissue regeneration. The current study has focused on the in vitro assessment of protein-based biomaterials that could be a potential source for corneal scaffolds. Collagen and soy protein isolate (SPI) were used to prepare films by compression molding, while solution casting was employed to prepare gelatin films cross-linked with lactose or citric acid. The physical characterization of the films showed transmittance values that met the light transmission needs of the cornea and the in vitro degradation profile revealed a progressive decomposition of the biomaterials in enzymatic and hydrolytic solutions, which could promote the simultaneous integration with the native tissue. Cell viability of human corneal epithelial cells (HCE) and 3T3 fibroblasts was above 70% when exposed to SPI and gelatin films, even after 72 h. Live/dead assays and SEM analysis demonstrated the adhesion of both cell types to the matrixes, with a very similar morphology and arrangement to that observed in controls. Besides, both cell lines were able to proliferate and migrate over the films to a cell-free area, simulating the cell repopulation that would happen in a wound. These results demonstrated that the studied biomaterials could be potential alternatives applicable in corneal bioengineering. Research study supported by grants from the Basque Government (RIS3, 2020333027) and UPV/EHU - ICQO (US19/18).

Abstract **#1815**

NEOVASCULARIZATION STIMULATING HYDROGELS FOR REGENERATIVE MEDICINE

Anna Schmidbauer$_P$[1,2,3], Raffael Reichsöllner[1,2,3], Ana Puljic[1,2,3], Johannes Hackethal[4,3], Heinz Redl[5,3], Stefan Baudis[1,2,3]

[1] *Christian Doppler Laboratory for Advanced Polymers for Biomaterials and 3D Printing, TU Wien*

[2] *Institute of Applied Synthetic Chemistry, TU Wien*

[3] *Austrian Cluster for Tissue Regeneration*

[4] *THT Biomaterials*

[5] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology*

Corresponding author's email: anna.schmidbauer@tuwien.ac.at

Introduction

Neovascularization of implants used as scaffolds is crucial for the regeneration of large tissue defects. Hence, the formation of vascular networks is of utmost importance. Photocrosslinkable hydrogels show promise as matrix for the ingrowth of neo-vessels. We integrated the mimicry of placenta, a highly vascularized organ, within gelatin (Gel)-hydrogels. Different Gel-modifications, including Gel-norbornene (Gel-NB) [1] and Gel-allyl glycidyl ether (Gel-AGE) [2] were in situ crosslinked enabling fabrication of complex structures either by extrusion-based 3D-bioprinting or by two-photo-polymerization.[3]

Methods

Gel was modified with 5-norbornene-2,3-dicarboxylic anhydride,N-hydroxysuccinimide-activated 5-norbornene-2-carboxylic acid (Gel-NBs), or allyl glycidyl ether (Gel-AGE) with degrees of modification. Hydrogel formation was monitored by photorheology using lithium phenyl-2,4-6-trimethylbenzoylphosphinate, as photoinitiator and D,L-dithiothreitol or ethoxylated trimethylolpropane tri(3-mercaptopropionate) as crosslinker in aqueous solution (2.5-10 wt%).[4].

Results

Mechanical properties (shear moduli 10-1000 Pa) and swellability (5-40 fold) of the hydrogels depend on the type/degree of modification. Hydrogel vascular network formation with HUVECs was observed when placenta-specific factors were incorporated.

Discussion and Conclusions

The formation of vascular networks could be observed with the prepared hydrogels. For the 3D fabrication of vascularization promoting hydrogel constructs, suitable system is to be found by variation of parameter (modification, crosslinker, gel content etc.).

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Abstract **#1816**

ENHANCED MECHANICAL PROPERTIES OF HARD-BLOCK BIODEGRADABLE THERMOPLASTIC POLYURETHANES FOR VASCULAR TISSUE REGENERATION

Philip Schwarzlᵽ[1,2], Katharina Ehrmann[1,2], Christian Grasl[3], Helga Bergmeister[4,2], Heinrich Schima[3], Bruno Podesser[4,2], Robert Liska[1,2], Stefan Baudis[1,2]

[1] *Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria*

[2] *Austrian Cluster for Tissue Regeneration, Austria*

[3] *Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria*

[4] *Center for Biomedical Research, Medical University of Vienna, Vienna, Austria*

Corresponding author's email: philip.schwarzl@tuwien.ac.at

Introduction

Biodegradable scaffolds for tissue engineering are getting more and more important, including vascular grafts with small-diameter.1 It is often reported, that materials like thermoplasts show good degradation behaviour. In contrast to that the mechanical properties are not sufficient for the intended application. Here, we introduced new hard-block degradable thermoplastic polyurethanes (TPUs) based on a variety of cleavable chain-extenders.2-4 These new materials provide new insights especially on the improvement of mechanical properties for thermoplasts.

Methods

TPUs, based on new developed cleavable chain-extenders, were processed via electrospinning, resulting in highly-porous tubes.2-4 The material itself was characterized in terms of biomechanical properties, degradability, biocompatibility and were tested in vivo.

Results

Different hard-block degradable TPUs were prepared by varying the hard-block components and by adjusting the ratio of these. These novel materials were compared in terms of mechanical properties, degradability and biocompatibility. There is a wide consensus that the mechanical properties are controlled by the hard-blocks and therefore by the choice of isocyanate and chain-extender. Since most biodegradable TPU concepts are based on ester-cleavage of soft-blocks, this concept was adapted for hard-block degradable TPUs by integration of cleavable chain-extenders. Furthermore, the mechanical properties of the materials were improved by introducing moieties, which also have the ability to form strong microcrystalline structures.

Discussion and Conclusions

During this study, we found a combination of TPU components, which results in small-diameter vascular grafts with superior mechanical properties, biocompatibility and degradability in vitro and promising performance in vivo.

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Abstract **#1817**

A COMPOSITE BIOMATERIAL-INK BASED ON HYALURONAN AND NANO HYDROXYAPATITE DELIVERING CHEMICALLY MODIFIED RNA FOR BONE REGENERATION

Daphne van der Heide[1], Florence de Groot-Barrère[2], Martin J. Stoddart[1], Matteo D'Este[1]

[1] *AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland*

[2] *Kuros Biosciences Bv, Professor Bronkhorstlaan 10, Building 48, 3723 MB Bilthoven, Netherlands*

Corresponding author's email: daphne.vanderheide@aofoundation.org

Most bone injuries heal without any complication, however, there is an increasing number of cases where bone lesions result in delayed healing or non-union. Current treatments such as autografting and bone graft substitutes containing growth factors have limitations, due to donor site morbidity and dose-related safety concerns, respectively. Additionally, current clinically available bone graft substitutes lack control of spatial architecture to anatomically match defect sites after complex fractures. The aim in this study was to engineer a biomaterial-ink composed of osteoinductive calcium phosphate and matrix biopolymers for delivery of chemically modified RNA inducing nerve, vessel and bone formation using 3D-printing. To this end, a 3D-printed matrix of tyramine modified hyaluronan (HA-Tyramine), combined with a range of nano-hydroxyapatite (nanoHA) particles, was developed. A parametric study comparing formulations with a range of 0-30% w/v nanoHA (size: 10-50x50-400 nm) showed that, the biomaterial-ink formulations of 3.5% w/v HA-Tyramine with 1 U/mL horseradish peroxidase (HRP), 0.17 mM (5.78 ppm) H2O2, and 0.02% w/v Eosin Y are extrudable using a 1.35 mm diameter nozzle. Extrusion of these formulations resulted in the formation of a continuous strut, with good shape retention and without waviness. Swelling and mechanical properties were dependent on nanoHA content. The range of compositions identified will be assessed for in vitro osteogenesis (cell viability, gene expression, protein production and mineralization) using human mesenchymal stem cells (hMSCs). To conclude, this study presents a 3D-printed biomaterial-ink with nanoHA that has the potential as a bone graft substitute for bone regeneration in trauma and osteoporotic patients.

Abstract **#1818**

ENGINEERED HUMAN BONE TISSUE USING ADIPOSE DERIVED STROMAL CELLS THROUGH ENDOCHONDRAL OSSIFICATION

Mansoor Chaaban[P][1,2], Adrien Moya[1,2], Thibaut Klein[1,2], Arnaud Scherberich[1,2]

[1] *Tissue Engineering Laboratory, Department of Biomedicine, University of Basel, Basel , Switzerland*

[2] *University Hospital Basel, Basel , Switzerland*

Corresponding author's email: mansoor.chaaban@unibas.ch

Stromal vascular fraction (SVF) cells, isolated from adipose tissue by enzymatic digestion, represent an attractive cell source for bone tissue engineering. They are easily accessible, abundant and include stromal, endothelial and hematopoietic cells[1]. Previous studies from our laboratory have shown the capacity of expanded-SVF cells to form bone through endochondral ossification (ECO)[2], but little is known about the specific mechanisms involved. Here, we studied the role of in vitro expansion, passaging and accessory cells in ECO by SVF cells.

We compared SVF cells and monolayer-expanded SVF cells, called adipose stromal cells (ASCs). Endothelial and hematopoietic cells were depleted from SVF-cells and ASCs with MACS microbeads. Thereafter, cells were cultured in collagen sponges with growth factors to generate cartilaginous templates (CT) in vitro or analyzed by mass spectrometry. SVF cells showed superior chondrogenic potential when compared to ASCs, as demonstrated by higher amounts of glycosaminoglycan (GAG) and by increased histological quality of the CT. The depletion of endothelial and hematopoietic cells showed limited effects on chondrogenesis. ASCs presented a highly different proteomic profile when compared to SVF cells, notably for pathways related to energy metabolism like glycolysis, TCA cycle and lipid metabolism.

This study evidences the role of metabolism in ECO by adipose-derived cells. Next steps will assess whether a more physiological cell culture environment would better preserve the specific metabolic status of SVF cells and thereby retain their chondrogenic and hypertrophic differentiation potential when expanded. Moreover, we will assess if better CT quality results in an improved remodelling in vivo.

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Abstract **#1819**

CONDITIONED MEDIUM FROM HUMAN AMNIOTIC MESENCHYMAL STROMAL CELLS TO MODULATE IMMUNOLOGICAL ALTERATIONS IN YOUNG AND OLD PATIENTS AFTER TRAUMATIC BRAIN INJURY

Marta Magatti[P][1], Anna Pasotti[1], Alice Masserdotti[1], Francesca Pischiutta[2], Enrico Caruso[2,3], Fabrizio Ortolano[3], Tommaso Zoerle[3,4], Marco Carbonara[3], Franco Capuzzi[5], Antonietta Silini[1], Elisa Zanier[2], Ornella Parolini[6,7]

[1] *Centro di Ricerca E.Menni, Fondazione Poliambulanza Istituto Ospedaliero, Brescia, Italy*

[2] *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy*

[3] *Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Dipartimento di Anestesia-Rianimazione e Emergenza Urgenza, Milano, Italy*

[4] *Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti*

[5] *Dipartimento Medicina di Laboratorio, Fondazione Poliambulanza Istituto Ospedaliero, Brescia, Italy*

[6] *Department of Life Science and Public Health, Università Cattolica del Sacro Cuore Facoltà di Medicina e Chirurgia, Roma, Italy*

[7] *Fondazione Policlinico Universitario "Agostino Gemelli" IRCCS, Roma, Italy*

Corresponding author's email: marta.magatti@poliambulanza.it

Traumatic brain injury (TBI) is the leading cause of mortality and disability among both young individuals and elderly subjects. Systemic inflammation has been shown to play a dominant role in TBI [1]. Furthermore, during aging, the immune system undergoes a functional priming towards a proinflammatory state that aggravates TBI outcome and response to therapy. Cells from the mesenchymal region of the amniotic membrane (hAMSC) and the conditioned medium generated from their culture (CM-hAMSC) act on cells of the immune system belonging to both innate (dendritic cells, monocytes and macrophages) and adaptive (T and B lymphocytes) immunity, controlling inflammation, proliferation, and cytokines production [2].

Considering the role of the immune system in TBI, we aimed to investigate the immunological changes that occur in peripheral blood mononuclear cells (PBMC) collected 6-24h after TBI in young (18-45 yo) and aged (>65 yo) patients, and how CM-hAMSC acts on these PBMC. Preliminary results show that PBMC from TBI patients have a significantly lower yield and viability after thawing when compared to age-matched controls. Moreover, TBI induced a significant reduction in the frequency of T cells, and an increase of monocytes, compared to healthy donors. Differences in the CD4/CD8 ratio, in the composition of Th subsets (Th1, Th2, Th17, Th22) and in memory T cell subsets (central and effector memory) were observed, and further studies are ongoing to determine the effect of CM-hAMSC on PBMC. Our results could help to better understand the contribution of systemic immune changes to TBI evolution and response to therapy.

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Abstract **#1820**

DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS INTO THE FUNCTIONING TRABECULAR MESHWORK CELLS

Emine Kubra Bilir[P][1], Xiaochen Fan[1], Rachel Oldershaw[2], Carl Sheridan[1]

[1] *Department of Eye and Vision Science, Institute of Life Course and Medical Sciences, University of Liverpool, UK*

[2] *Department of Musculoskeletal Biology I, Institute of Life Course and Medical Sciences, University of Liverpool, UK*

Corresponding author's email: ekbilir@liverpool.ac.uk

INTRODUCTION

Trabecular Meshwork (TM) cells serve as a biological filter by phagocytosing debris accumulated in aqueous humor. The number of TM cells decreases with age and more so in the Primary Open Angle Glaucoma. A cell-based functional restoration of TM, therefore, represents a new therapeutic approach. This study examines different protocols in the ability of induced Pluripotent Stem Cells (iPSCs) to differentiate into functioning TM cells.

METHODS

The following four methods I) TM cell-conditioned media (CM), II) Co-culturing iPSCs with TM cells using a transwell membrane, III) Co-culturing iPSCs with TM cells using the double surface of transwell membrane IV) Culturing iPSCs on TM Extracellular Matrix (ECM) with TM-CM were used to differentiate peripheral blood mononuclear and fibroblast dermis cells originated two human iPSC lines.

RESULTS

Culturing iPSCs on TM Extracellular Matrix (ECM) with TM-CM was identified as the best method for differentiation of iPSCs into TM cells. The differentiated cells demonstrated TM morphological characteristics (elongated, spindle-shaped) and gene expression of specific TM cell markers, Chi3l1, AQP1 and MGP significantly greater than to iPSCs ($p<0.005$) but not different than control TM cells ($p>0.05$). Differentiated iPSCs had undetectable expression levels of pluripotency genes; Sox2 and Oct4.

DISCUSSION

The study suggests that the TM ECM plays an important role in differentiating human iPSCs into TM cells. Generating a source of iPS derived TM cells has the potential for future drug screening or transplant approaches.

CONCLUSION

iPSCs became differentiated to TM-like cells after sustained exposure to ECM of TM cells and CM together.

Keywords

Induced Pluripotent Stem Cells; Trabecular Meshwork Cells; Glaucoma

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Abstract **#1821**

MINIMAL NON-MARKOVIAN COMPUTATIONAL MODEL TO FURTHER UNDERSTAND POLYMERIC BIOMATERIALS PROPERTIES AROUND THE GLASS TRANSITION

Jose Luis Aparicio Collado[P][1], Constantino Torregrosa Cabanilles[1], José Molina Mateo[1], Roser Sabater i Serra[1,2], José María Meseguer Dueñas[1], José Luís Gómez Ribelles[1,2]

[1] Centre for Biomaterials and Tissue Engineering, Universitat Politècnica de València, 46022 València, Spain

[2] CIBER-BBN, Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine, 46022 València, Spain Spain

Corresponding author's email: joapcol@upvnet.upv.es

The glass transition temperature of a polymer is one of the most important parameters to consider in the development of new polymeric biomaterials or understanding their behavior. It determines its use temperature, physical and mechanical properties, and material processing conditions. However, it still remains one of the most significant unsolved problems in condensed matter physics [1].

Computer simulations provide many important insights at the molecular level in materials science, offering significant new knowledge about the performance of polymeric biomaterials [2]. In this context, computer simulations can be considered as a bridge that connect microscopic dynamics (such as interaction between atoms or molecular geometry) to macroscopic properties of polymeric systems such as the glass transition temperature, diffusion coefficient or viscosity [3].

The complex behavior of the material around the glass transition has been reproduced by a Non-Markovian computational model, reducing the system under study to a nanoscale region formed by a cluster of a small number of molecular groups coupled to their environment. Dynamic heterogeneity was implemented in this model through the existence of slow and fast regions which can change randomly with two different potential barrier heights [4]. This minimal stochastic model developed for small open regions reproduces the main phenomena found in the glass transition and can be useful as a basis for the development of more specific models that allow understanding the complex behavior underlaying the glass transition and structural relaxation processes.

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Abstract **#1822**

DEVELOPMENT OF HYBRID HYDROGEL SCAFFOLDS FOR LIVER TISSUE ENGINEERING

Nathan Carpentier$_P$[1], Lindsey Devisscher[2], Hans Van Vlierberghe[3], Peter Dubruel[1], Sandra Van Vlierberghe[1]

[1] Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Ghent, Belgium

[2] Gut-Liver Immunopharmacology Unit, Dpt Basic and Applied Medical Sciences; Liver Research Center Ghent; Ghent University, Ghent, Belgium

[3] Hepatology Research Unit, Dpt Internal Medicine and Paediatrics; Liver Research Center Ghent; Ghent University, Ghent, Belgium

Corresponding author's email: nathan.carpentier@ugent.be

Introduction:

Annually, millions of people die because of liver failure, while the waiting duration for a donor liver is around 12 months.[1][2] Here, we developed hybrid 3D-printed scaffolds to serve liver tissue engineering(LTE) applications. Gelatin and dextran were used as starting material, crosslinked using two different chemical approaches to explore their effect on cell response and GelMA served as benchmark[3].

Materials and methods:

Thiolated gelatin(GelSH)[4] was combined with norbornene-functionalized dextran(DexNB)[5] followed by a radical step-growth thiol-NB crosslinking. In parallel, oxidized dextran(Dexox) and gelatin were crosslinked using a Schiff-base mechanism. Both materials were characterized on 2D-and 3D-level and compared with GelMA regarding their physico-chemical properties (swelling, gel fraction, mechanical properties) and in vitro biocompatibility (cell proliferation, live-dead staining). The 3D-scaffolds were developed using indirect 3D-printing[6].

Results and discussion:

On a 2D-level, GelMA and DexNB-GelSH were superior over Dexox-gel as their crosslinking kinetics were significantly faster and they mimicked natural liver tissue (NLT) to a greater extent with respect to swelling and mechanical properties.

On a 3D-level, DexNB-GelSH scaffolds exhibited a compressive modulus of 4.8±1.6kPa which is in excellent agreement with that of NLT(i.e. 1–5kPa)[7] as compared to GelMA which resulted in a modulus of 8.5±1.9kPa. However, the in vitro biocompatibility of both materials was comparable.

Conclusions:

DexNB-GelSH scaffolds are promising hybrid materials to support LTE as they exhibit similar physico-chemical properties compared to NLT (cfr. compressive modulus and swelling ratio), while cell viability and proliferation were preserved. Further analyses evaluating the preservation of cell functionality are ongoing.

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Abstract **#1823**

SIMULATION OF MECHANICAL STRENGTH OF BIOACTIVE WHITLOCKITE/DIOPSIDE GLASS-CERAMIC IMPLANTS USING FINITE ELEMENT METHOD (FEM)

Manuel Fellipe Rodrigues Pais Alves[P][1], Maria Helena Figueira Vaz Fernandes[1], Juliana Kelmy Macário Barboza Daguano[2], Claudinei Dos Santos[3], Pedro Araújo da Costa Ward[3], Luciano Pessanha Moreira[3]

[1] CICECO – Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, Aveiro, 3810-193, Portugal

[2] Universidade Federal do ABC – Centro de Engenharia, Modelagem e Ciências Sociais Aplicadas, Alameda da Universidade, s/n - Anchieta, São Bernardo do Campo - SP, Brazil

[3] Universidade Federal Fluminense- Escola de Engenharia Industrial de Volta Redonda, Vila Santa Cicília, Volta Redonda-RJ, Brazil

Corresponding author's email: manuel.alves@ua.pt

Glass-ceramics based on the CaO–P2O5–SiO2–MgO system have presented biocompatible phases as hydroxyapatite (HA), tricalcium phosphate (TCP), and TCP with magnesium substituting partial calcium (TCMP). The β-TCMP phase with whitlockite structure has interesting biological features and mechanical properties, meeting the bioactivity requirements for bone restoration. In this work, a 3CaO·P2O5-SiO2-MgO glass was submitted to different heat treatments of 4h at temperatures from 775 to 1100 ℃. After that, samples were mechanically characterized. It was observed a direct correlation between the induced crystalline phases and the increase of temperature treatment, resulting in a rise of bending strength (70 – 120MPa). Likewise, the elastic parameters were determined (Elasticity modulus = 92 – 162 GPa, Poisson's ratio = 0.26 – 0.27). From that, numerical simulation using the finite element method (FEM) was proposed to interpret the mechanical response of these bioactive glass-ceramic implants' as a function of its intrinsic geometry parameters and the application of masticatory loads. A geometry implant model of Ø4.1 - 4.8 mm, 0.8 mm thread pitch, containing 11,146 knots (45,286 elements), and a bone structure (cortical + spongy) with 31,200 knots were developed using CATIA V5 software. These models were used to simulate human adult male mandible, implanted in the third molar region, through the HyperWorks and Abaqus software. Finally, loads between 100 – 300N were simulated using FEM at different application angles, replicating an adult's effort. Results were discussed according to the crystalline phases, the amount of residual glass, and normative requirements for the use of dental implants.

Keywords

Dental implants; Glass-ceramics; FEM simulation

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Abstract **#1825**

BIOLOGICAL EVALUATION OF (CE,Y)-TZP/AL2O3 CERAMIC COMPOSITES FOR DENTAL IMPLANTS WITH DIFFERENT ROUGHNESS

Manuel Fellipe Rodrigues Pais Alves[P][1], Maria Helena Figueira Vaz Fernandes[1], Andrea Cecilia Dorión Rodas[2], Juliana Kelmy Macário Barboza Daguano[2], Claudinei Dos Santos[3]

[1] CICECO – Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, Aveiro, 3810-193, Portugal

[2] Universidade Federal do ABC – Centro de Engenharia, Modelagem e Ciências Sociais Aplicadas, Alameda da Universidade, s/n - Anchieta, São Bernardo do Campo - SP, Brazil

[3] Universidade do Estado do Rio de Janeiro - Faculdade de Tecnologia, Rod. Presidente Dutra, km 298, Resende-RJ, Brazil

Corresponding author's email: manuel.alves@ua.pt

Ceramic composites based on (Ce, Y)-TZP/Al2O3 system have great potential for applications as dental implants due to their excellent mechanical properties associated with high resistance to hydrothermal degradation. In this work, hydrothermal degradation and preliminary biological evaluation of a ceramic composite of the CeO2-Y2O3-ZrO2-Al2O3 system were investigated as a function of surface roughness. Compacted samples were sintered at 1500 °C - 2h and then submitted to different surface treatments: Group 1 composed of samples with smooth surfaces, Group 2 composed of rough surfaces (sanding with 45 µm diamond sandpaper). Samples were characterized by X-ray diffraction and optical profilometry and them subjected to hydrothermal degradation tests in autoclave (134 °C - 2 bar) for a maximum of 10h, using artificial saliva. To assess indirect cytotoxicity, samples were immersed in the culture medium for NIH-3T3 cells for 72 hours. Furthermore, cell adhesion and proliferation were investigated using MG63 cells (human osteosarcoma) after 3, 7, 14, and 21 days of culture. Cytotoxicity, adhesion, and cell proliferation were examined by the Methyl Tetrazolium salt (MTS) and Alizarin Red, using a confocal laser microscope. The results indicated that the materials have high resistance to degradation, having low monoclinic phase concentrations only on surfaces with high roughness (45 µm). The (Ce, Y)-TZP/Al2O3 composites are not cytotoxic. The rougher sample shows the best cellular adhesion and proliferation, leading to a mineralized matrix formation after 21 days. These results clearly suggest that the new (Ce, Y)-TZP/Al2O3 brand is strong and highly biocompatible and warrants further study.

Keywords

(Ce,Y)-TZP/Al2O3 composite; Hidrothermal degradation; Biological characterization

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Abstract **#1827**

THE EFFECT OF DIFFERENT MOLAR RATIOS OF PEGYLATED FIBRIN ON ISOLATED OVARIAN STROMAL CELLS

Arezoo Dadashzadeh$_P$[1], Saeid Moghassemi[1], Christiani Andrade Amorim[1]

[1] Pôle de Recherche en Gynécologie, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, 1200 Brussels, Belgium

Corresponding author's email: arezoo.dadashzadeh@uclouvain.be

This study aimed to assess the influence of two formulations of PEGylated fibrin on the survival and proliferation of ovarian stromal cells. To this end, a total of 2.4×104 cells were equally divided into two groups according to the molar ratios of PEGylated fibrinogen (PEG:Fib): 10:1 and 5:1. After polymerization with 50 IU/ml thrombin, the cells embedded in the hydrogels were in vitro cultured for five days. Histological and immunohistological analysis of hydrogels before (day 0) and after in vitro culture was performed. Cell density dynamic (Cd) was calculated by the following formula:

Cd= (density of day 5-density of day 0)/(density of day 5)×100

Our results show that while more elongated cells were observed in the PEG:Fib 5:1 hydrogel, rounded shape cells were more often found in the other group (Fig. 1). This could be due to the higher availability of bioactive sites in PEG:Fib 5:1, which fewer PEG molecules have interacted with the fibrin domains. The average proportions of Ki67-positive cells were high in PEG:Fib 10:1 and 5:1 groups, around 24% and 22%, respectively. Additionally, more caspase 3-positive cells were also seen in also appeared PEG:Fib 10:1 scaffold. On the other hand, PEG:Fib 5:1 indicated a considerable Cd compared to PEG:Fib 10:1 (Fig. 2). In conclusion, increasing the molar ratio of PEG:Fib can decrease the availability of bioactive sites of fibrinogen, which may be masked or bound by PEG molecules and affect the cellular behaviors in terms of proliferation, apoptosis, and morphology.

Keywords

Ovarian tissue engineering; PEGylated fibrin; Hydrogel

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Abstract **#1828**

ROLE OF NON-CANONICAL WNT11 FOR ENDOCHONDRAL DIFFERENTIATION OF HUMAN MESENCHYMAL STROMA CELLS

Malina Seguin[P][1], Safak Chasan[1], Wiltrud Richter[1], Moritz Innmann[2]

[1] *Research Centre for Experimental Orthopaedics, Orthopaedic University Hospital Heidelberg, Heidelberg, Germany*

[2] *Department of Orthopaedic and Trauma Surgery, Orthopaedic University Hospital Heidelberg, Heidelberg, Germany*

Corresponding author's email: Malina.Krueger@med.uni-heidelberg.de

Human mesenchymal stroma cells (MSC) are a promising cell source for generation of engineered cartilage. However, during in vitro differentiation MSCs develop along the endochondral pathway and become hypertrophic, which is undesired for cartilage regeneration. The signaling mechanisms regulating hypertrophic differentiation are not fully understood. We recently demonstrated during in vitro chondrogenesis of MSCs, expression of non-canonical WNT11 increased along with hypertrophic markers, while no typical canonical WNT ligand was found to be upregulated. Aim of this study was to illuminate the role of non-canonical WNT11 for hypertrophic differentiation of MSCs compared to a canonical WNT ligand. Understanding the role of canonical and non-canonical WNT signaling for endochondral differentiation, will allow improved articular cartilage neogenesis suitable for clinical regeneration of stable cartilage tissue. MSC in vitro chondrogenesis was treated with recombinant WNT11 protein, canonical WNT3A or the canonical WNT-antagonist DKK1 from day 14 on. Chondrogenic and hypertrophic markers were assessed on mRNA and protein levels on day 28. WNT3A-stimulation enhanced expression of hypertrophic markers MEF2C, ALPL, IBSP and IHH at maintained COL2A1 levels. Enhanced ALP enzyme activity and higher IBSP protein levels confirmed the pro-hypertrophic action. Accordingly, DKK1-treatment significantly suppressed hypertrophic markers, demonstrating that canonical WNT signaling is driving hypertrophy. WNT11-stimulation reduced ALPL expression and ALP enzyme activity only slightly but significantly. In conclusion, non-canonical WNT11 is no main driver of hypertrophy during chondrogenic differentiation of hMSCs. Excessive canonical WNT signaling, from a yet unidentified ligand, is the critical factor driving hypertrophic differentiation of hMSCs and preventing stable chondrogenesis.

Abstract **#1830**

CELL MORPHOLOGY AS A DESIGN PARAMETER IN THE TISSUE ENGINEERING OF CELL-SURFACE INTERACTIONS.

Oliver Frost$_P$[1], Elena García-Gareta[1,2], Nazanin Owji[1,2], Prasad Sawadkar[1,3], Ferdinand Lali[4,3], Christos Kyriakidis[1]

[1] *Regenerative Biomaterials Group, The RAFT Institute & The Griffin Institute, Northwick Park & Saint Mark's Hospital, London, UK*

[2] *Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, London, UK*

[3] *Division of Surgery and Interventional Science, University College London, London, UK*

[4] *The Griffin Institute, Northwick Park & Saint Mark's Hospital, London, UK*

Corresponding author's email: oliver.frost7@gmail.com

Control of cell-surface interaction is necessary for tissue engineering applications such as cell sheets, intelligent cell culture surfaces, or functional coatings. The cell morphology measured through cell aspect ratio (CAR) can indicate ideal candidates for these various applications thus reducing the time taken for the screening and development process. The cell aspect ratio of primary porcine dermal fibroblast (PDFs) (3 biological replicates) were tested on ten different surfaces comprising components of the natural extracellular matrix of tissues. Characterisation followed with proliferation and cell migration assays, which clearly highlighted an optimal morphology for increased cell proliferation and migration. If the CAR was 0.1 (very elongated cell), the cell migration and proliferation were extremely reduced compared to those in 0.2-0.3 CARs. The same was evidenced in PDFs with a CAR of 0.4+ (very rounded cell). These results suggest a prime cell shape for PDFs to migrate and proliferate, which is a key aspect in tissue engineering applications requiring control of cell-surface interaction. This project shows the beneficial use of testing the cell morphology on prospective prototypes for screening, eliminating those that do not support an optimal cell shape. In addition, the technique used to calculate CAR provides a useful, simple alternative to monitor morphology if DAPI staining is not available.

Keywords

Cell Morphology; Cell Migration; Cell-Material Interaction

Abstract **#1831**

COMPARISON OF EXTRACELLULAR VESICLE ISOLATION PROCESSES FOR APPLICATIONS IN SKELETAL MUSCLE TISSUE ENGINEERING

Soraya Williams[P][1], Maria Fernandez-Rhodes[1], Alice Law[2], Ben Peacock[2], Mark Lewis[1], Owen Davies[1]

[1] *School of Sport, Exercise and Health Sciences, Loughborough University*

[2] *NanoFCM Co.Ltd, Medicity, Building D6, Thane Road, Nottingham, NG90 6BH, UK*

Corresponding author's email: s.williams3@lboro.ac.uk

Evidence suggests skeletal muscle (SkM) myotube derived extracellular vesicles (EVs) drive SkM processes, indicating their regenerative potential. However, therapeutically aligned studies are limited by the ability to efficiently obtain high yield purified populations from minimal sample volumes in a cost-effective manner. This study compared isolation methods to understand EV output variation using Nanoparticle tracking analysis (NTA), BCA protein assay, western blots (CD9, CD63, Alix, Annexin A2), ExoELISA's (CD63 and CD81) and nano flow cytometry (NanoFCM) (CD9, CD63, CD81). To further understand advantages and limitations (e.g. efficiency, cost, scalability, purity) a survey was internationally distributed via Qualtrics within the EV community. EVs were isolated from C2C12 mouse myoblast cells by ultracentrifugation (UC), polyethylene glycol (PEG) precipitation, Total Exosome Isolation Reagent (TEIR), an aqueous two-phase system (ATPS) utilising PEG and dextran, and size exclusion chromatography (SEC). ATPS was repeated with multiple washes of the top PEG phase for additional purification. TEIR displayed the highest particle concentration (1.15E+09) followed by UC (8.46E+08) and PEG precipitation (7.11E+08), with purity (particles per µg protein) 7.16E+05, 1.22E+06 and 6.90E+05 respectively. SEC and ATPS showed lower sample purity, 1.12E+05 and 1.99E+05 and particle counts of 4.57E+08 and 6.31E+08 respectively. ATPS with repeat washes displayed the lowest particle counts (2.71E+08) and highest purity (2.01E+06). EV markers were present in all isolations but with distinctly variable profiles. This high-throughput comparison study indicates that methods withdraw myogenic EV-enriched fractions with variable purity and marker profiles. This could have significant implications for defining SkM processes and EV applications in tissue engineering.

Abstract **#1832**

INVERSE EFFECTS OF CALCIUM ON CARTILAGE MATRIX PRODUCTION BY ARTICULAR CHONDROCYTES VERSUS MESENCHYMAL STROMA CELLS: IMPLICATIONS FOR OSTEOCHONDRAL CARTILAGE ENGINEERING

Tim Hammersen[P][1], Severin Zietzschmann[2], Wiltrud Richter[1]

[1] *Research Center for Experimental Orthopaedics, Heidelberg University Hospital, Heidelberg, Germany*

[2] *Clinic for Orthopaedics and Trauma Surgery, Heidelberg University Hospital, Heidelberg, Germany*

Corresponding author's email: tim.hammersen@med.uni-heidelberg.de

Purpose: Treatment of articular cartilage lesions often reveals mechanical failure at the graft-to-host tissue junction. Osteochondral tissue-engineering (TE) constructs composed of a resorbable calcified bone-substitute with a cartilage-mimetic scaffold on top showed better tissue integration and mechanical stability than non-zonal repair strategies. However, little is known about how elevated calcium ion-release from a bone-substitute affects differentiation of chondrogenic cells and whether articular chondrocytes (AC) or mesenchymal stroma cells (MSC) are more sensitive to such conditions. Aim of this study was to compare the influence of elevated [Ca2+] on differentiation and cartilage matrix production of AC and MSC to identify the ideal cell source for zonal cartilage TE.

Methods: Human AC and MSC were seeded in collagen type I/III scaffolds, cultured for 35 days under chondrogenic conditions at standard (1.8mM) or elevated (8.0mM) extracellular CaCl2 and assayed for GAG and collagen-type II production by radiolabel-incorporation, ELISA, qPCR and Western blotting.

Results: In cartilage engineered from ACs, elevated calcium significantly reduced GAG-synthesis, GAG/DNA and collagen-type II deposition and downregulated chondrogenic marker gene expression. Opposite, calcium significantly upregulated GAG- and collagen-deposition in the MSC group where chondrogenic and hypertrophic markers remained unchanged. Selective PTHrP-induction by calcium-treatment in AC suggests their enhanced sensitivity to calcium via the calcium-responsive PTHrP pathway compared to MSC.

Conclusion: Overall, it is important to consider the origin of the chondrogenic cell source for multilayered cartilage TE and we propose to separate an AC-layer from calcified structures by an intermediate layer of MSC.

Abstract **#1833**

HIGH-THROUGHPUT PRODUCTION OF HIPSC-DERIVED VASCULARIZED MYOGENIC SPHEROIDS FOR 3D BIOPRINTING

Jasper Smet$_P$[1], Heidi Declercq[1], Lieven Thorrez[1]

[1] *Tissue Engineering Lab, Department of Development and Regeneration, Faculty of Medicine, KU Leuven, campus Kulak, E. Sabbelaan 53, 8500 Kortrijk, Belgium*

Corresponding author's email: jasper.smet@kuleuven.be

Despite decades of progress, tissue engineering (TE) remains severely hampered by the incompetence to generate complex histoarchitectural features including vascular networks (1).

The 3D bioprinting of spheroids is ideally positioned to overcome these obstacles since it allows the directed assembly of microtissues that resemble the target tissue structurally and functionally (2). Therefore, we previously employed a high-throughput non-adhesive agarose microwell system for the generation of printable vascularized spheroids comprising adult endothelial cells, fibroblasts and adipose tissue-derived mesenchymal stem cells (MSCs) (3). These spheroids could fuse and inosculate their individual capillary-like networks (3) and confirmed their potential as microvascular building blocks in 3D extrusion printing (publication in preparation). We intend to exploit this strategy for the engineering of a human induced pluripotent stem cell (hiPSC)-derived vascularized muscle model. Therefore, we now created vascularized myogenic spheroids composed of hiPSC-derived ECs (Stem Cell Institute, KU Leuven), hiPSC-derived MSCs (Cellular Dynamics) and hiPSC-derived myoblasts (iXCells Biotechnologies). Upon application of the most optimal coculture ratio, the cells managed to form uniform and viable spheroids with dimensions compatible with 3D extrusion printing (Ø around 130 µm). Immunohistochemical, transmission electron microscopic and confocal fluorescence microscopic analyses indicated that the ECs were able to sort into capillary-like networks. Similar to our previous experiments, the presence of hiPSC-derived MSCs appeared indispensable for an optimal spheroid stability and vascularization. In future work, these vascularized microtissues will be implemented in 3D bioprinting to fulfill the need for vasculature within the low-micron range (<10 µm) in our muscle model.

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Abstract **#1834**

COLLAGEN-GLYCOSAMINOGLYCAN & RECOMBINANT-HUMAN-PROTEOGLYCAN-4 SCAFFOLDS FOR CARTILAGE REPAIR

Austyn R Matheson[P][1], Eamon J Sheehy[1,2,3], W Michael Scott[4], Tannin A Schmidt[5], Fergal J O'Brien[1,2,3]

[1] *Tissue Engineering Research Group, Royal College of Surgeons in Ireland, Dublin, Ireland*

[2] *Trinity Centre for Biomedical Engineering, Trinity College Dublin, Dublin, Ireland*

[3] *Advanced Materials and Bioengineering Research Centre, Trinity College Dublin, Dublin, Ireland*

[4] *University of Calgary, Calgary Canada*

[5] *University of Connecticut, Health Campus, Farmington, U.S.A.*

Corresponding author's email: austynmatheson@rcsi.com

Introduction

The efficacy of tissue-engineered (TE) cartilage scaffolds(1,2,3), an alternative to traditional cartilage repair techniques, can be augmented by adding tissue-specific matrix-components(3,2,4) and growth factors(5) to biomaterials. Proteoglycan-4, PRG4, is a superficial-zone cartilage protein and a synovial fluid constituent, with critical roles in boundary lubrication and joint homeostasis(6,7,8,9). Intra-articular supplementation of PRG4 is chondroprotective(10,11,12,13), shown to preserve cartilage volume(10,11), improve damage scores(12), and maintain biomechanics(13) in models of injury. Therefore, the hypothesis of this study was that the addition of PRG4 to TE cartilage scaffolds would enhance the lubricating function of biomaterials and therefore healing potential.

Methods

Recombinant-human (rh)-PRG4 was provided by Lμbris Biopharma(14). Collagen-glycosaminoglycan (coll-GAG) scaffolds were prepared by lyophilization(15) of a collagen I, hyaluronan, and rhPRG4 slurry at varying concentrations. Mesenchymal stem cells were cultured on collagen-GAG scaffolds. Biological assessments included: proliferation (Quant-iT PicoGreen), metabolic activity (alamarBlueTM), GAG synthesis (Blyscan, Biocolor), and cell infiltration (immunohistochemistry: hematoxylin & eosin, safranin-o). The coefficient of friction (COF) of scaffolds in PBS was characterized with an adapted scaffold-on-glass test(16,17,18).

Results

Coll-GAG-rhPRG4 scaffolds had significantly reduced COF compared to controls (0.068 ±0.01 vs. 0.118±0.02, $p<0.001$), and decreased compressive stiffness. The rhPRG4 released from scaffolds was intact (460kDa) and protein released completely from materials in seven days. The addition of rhPRG4 to Coll-GAG scaffolds did not negatively effect cell proliferation or metabolism.

Discussion & Conclusion

rhPRG4 added to Coll-GAG scaffolds through bulk-incorporation had significantly reduced friction and gradual protein release times. The Coll-GAG-rhPRG4 material is a biomechanically-enhanced biomaterial for cartilage repair application.

Keywords

Collagen-Glycosaminoglycan; Proteoglycan-4; Cartilage

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Abstract **#1836**

BIOMIMETIC HYALURONIC ACID-BASED HYDROGELS TO INVESTIGATE ROLE OF TUMOR BIOPHYSICAL PROPERTIES ON GLIOBLASTOMA (GBM) PROGRESSION

Alireza Sohrabi[P][1], Jesse Liang[1], Austin Lefebvre[2], Michael Condro[1], Harley Kornblum[1], Michelle Digman[2], Stephanie Seidlits[1]

[1] *University of California, Los Angeles (UCLA)*

[2] *University of California, Irvine (UCI)*

Corresponding author's email: asohrabi@g.ucla.edu

Glioblastoma (GBM) is the most common, lethal type of brain cancers with 5-years survival of 5%. The unique microenvironment of GBM is one of the main factors in tumor aggression. Specifically, similar to other solid tumors, GBM cells experience different biomechanical forces than cells in a healthy brain tissue. Our micro-compression mechanical analysis (using AFM) of GBM tumors shows a stiff core (5-6 kPa Young's modulus), a softer tumor edge (1-2 kPa Young's modulus), a very soft non-tumor brain tissue (0.5-0.8 kPa Young's modulus). To understand how this biomechanical landscape affects GBM cells, GBM spheroids were encapsulated in biomimetic hyaluronic acid-based (HA) hydrogels, mimicking stiffness of a tumor and neighboring brain tissue. In soft hydrogels, GBM cells migrate away from original spheres whereas we did not witness any migration in stiff hydrogels. RNA-sequencing analysis revealed overexpression of mitochondrial genes in stiff conditions, involved in oxidative phosphorylation (OXPHOS), when compared to soft hydrogels. This data suggests GBM cells in soft hydrogels undergo aerobic glycolysis (GLY). We then performed fluorescence-lifetime imaging microscopy (FLIM) to measure amount of bound NADH (OXPHOS) vs. unbound NADH (GLY). FLIM data revealed higher presence of bound NADH in GBM cells encapsulated in stiff hydrogels which in turn suggests more OXPHOS metabolic activity. On the other hand, more unbound NADH was observed in soft hydrogels suggesting more GLY metabolic activity in soft hydrogels. In sum, we find that a softer microenvironment (like normal brain) promotes GBM cells migration and a switch to a more malignant metabolic pathway.

Abstract **#1837**

BONE MARROW SUPERNATANT FOR MSC SUPPLEMENTATION ELIMINATES IMMUNE TARGETING

Aileen Rowland$_P$[1], Madison Burns[1], Gwendolyn Levine[2], Ashlee Watts[2]

[1] *Texas A&M University*

[2] *Texas A&M University*

Corresponding author's email: arowland@cvm.tamu.edu

Market authorization of mesenchymal stromal cells (MSCs) has yet to be achieved in the United States, and lack of regulatory approval could be due to MSC preparation techniques. Fetal bovine serum (FBS) has been used for MSC preparation in recent US clinical trials and also for MSC therapeutics with current foreign market authorization. Likewise, FBS is almost exclusively used in animal models. We hypothesize that the use of FBS results in recipient immune targeting of therapeutic MSCs, thereby preventing their clinical effect. In the equine model, we evaluated the effect of FBS in MSC preparation by performing repeated intra-articular injection of MSCs cultured with and without FBS. First, we validated the use of bone marrow supernatant (BMS) in vitro as a replacement for FBS, and noted an increased rate of MSC isolation but no difference in MSC expansion or characteristics. Supplementation of MSC with FBS caused local inflammation, antibody mediated MSC cytotoxicity, and reduced synovial MSC concentration, despite a lack of change in anti-bovine antibodies in recipients. These adverse effects and immune targeting were ameliorated with the replacement of FBS with BMS. For the first time, we show that FBS supplemented MSCs are targeted by the recipient immune system resulting in local inflammation, cytotoxicity, and reduced efficacy. Fetal bovine serum should no longer be utilized in clinical study, and the use of FBS in pre-clinical and historical clinical studies should be considered when interpreting results. Bone marrow supernatant as an FBS replacement should be further investigated.

Abstract **#1839**

DIFFERENTIAL REGULATION OF PI3K/AKT SIGNALING BETWEEN ENDOCHONDRAL DIFFERENTIATION OF MESENCHYMAL PROGENITOR CELLS AND NON-HYPERTROPHIC MATURATION OF ARTICULAR CHONDROCYTES

Felicia Anna Maria Klampfleuthner$_P$[1], Benedict Lotz[2], Wiltrud Richter[1], Solvig Diederichs[1]
[1] *Research Center for Experimental Orthopaedics, Heidelberg University Hospital, Heidelberg, Germany*
[2] *Clinic for Orthopaedics and Trauma Surgery, Heidelberg University Hospital, Heidelberg, Germany*
Corresponding author's email: felicia.klampfleuthner@med.uni-heidelberg.de

Introduction: Re-directing mesenchymal progenitor cell (MPC) chondrogenesis towards a non-hypertrophic articular chondrocyte-(AC)-like phenotype is important for facilitating cartilage repair with MPC. PI3K/AKT signaling promotes proteoglycan-deposition and chondrocyte hypertrophy in the growth plate. AKT inhibition during early MPC chondrogenesis suppressed proteoglycan-deposition according to our previous data, but later AKT inhibition and effects on hypertrophy remained uninvestigated. Aim was to uncover PI3K/AKT-pathway activity during chondrogenic (re-)differentiation of MPC and AC and illuminate functional consequences.

Methods: Human expanded MPC and AC were subjected to standard chondrogenic pellet culture and assayed for expression of AKT-related genes at day 28 as well as pAKT/AKT pathway activity via Western-blotting at day 1, 3, 7 and at weekly intervals until day 42. Differentiation was assessed histologically and via DMMB-assay.

Results: MPC started into chondrogenesis with significantly lower pAKT and AKT levels and expressed AKT-activators PDPK1, JUN, JUNB, JUND, STAT3 and AKT1 lower, but the AKT-inhibitor PTEN higher than AC. While AC rapidly silenced AKT-activity at switch to differentiation culture, AKT pathway activity raised in MPC parallel to hypertrophic markers reaching significantly higher pAKT levels from d21 on. In line, AKT-related PDPK1, JUNB and JUND expression was significantly enhanced compared to AC. Late AKT suppression during MPC chondrogenesis from d21 on reduced SOX9 protein levels, SMAD2/3 signaling and chondrogenic differentiation.

Conclusion: Selective upregulation of AKT-pathway activity only in MPC parallel to hypertrophy suggests its relevance for MPC mis-differentiation. Crosstalk of the AKT-pathway with TGFβ signaling is currently followed up as a putative mechanism of potential pro-hypertrophic AKT action.

Keywords
mesenchymal progenitor cells; hypertrophy; AKT

Abstract **#1840**

ASSESSING CONTRACTILITY OF 3D IPSC-DERIVED ENGINEERED MUSCLE TISSUES AT HIGH-THROUGHPUT USING A NOVEL, LABEL-FREE METHOD

Nicholas A Geisse[P1], Bonnie J Berry[1], Travis Moerk[1], Eli J Fine[1], Jason Silver[1], Kevin Gray[1], Samir Kharoufeh[1], Nathan J Sniadecki[2], David Mack[2]

[1] *Curi Bio*

[2] *University of Washington*

Corresponding author's email: heejoon@curibio.com

Preclinical assays often fail to predict drug action, resulting in high clinical trial failure rates. Cellular models can screen for cardiotoxicity, but oftentimes utilize complex 3D systems that—while predictive—are challenging to deploy at sufficient scale. Widespread adoption of engineered muscle tissues (EMTs) has been hindered by traditional fabrication methods that require extensive bioengineering and by measurement methods that involve low-throughput approaches. We designed, optimized, and validated a novel method to fabricate and measure the contractility of 24 3D EMTs in parallel.

Our tissue casting approach improves success rate to >95% (n > 30) and produces consistently-sized constructs with a standard deviation of +/- 9% of the mean across 6 experiments. The substrate features an embedded magnet; as tissues contract, the magnet's displacement is quantitatively detected in a highly-parallel manner using specialized sensors. We detected 24 contractions simultaneously with a rate of measurement 100Hz, which is suitable for measuring various aspects of contractility such as upstroke velocity and decay time.

This method can measure drug-induced changes to EMT contractility, such as doxorubicin, a structural cardiotoxicant. Treated EMTs showed no differences over controls at acute (30 min) or daily timepoints to Day 2 post exposure. The highest dosed (1 μM) tissue began to slow at Day 4 ($p < 0.01$) and Day 5 ($p < 0.0001$) until complete cessation of twitching by Day 6. We will also present data showing phenotype stratification across EMTs from healthy and diseased individuals, demonstrating the potential of the platform to be used in drug discovery studies.

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Abstract **#1841**

BIOPHYSICAL REGULATION OF IN-VITRO GASTRULATION USING INDUCED PLURIPOTENT STEM CELLS

Pallavi Srivastava$_P$[1,2], Sara Romanazzo[2], Jake B Ireland[2], John E Pimanda[3,1], Kristopher A Kilian[2,4]

[1] *School of Medical Sciences, University of New South Wales, Sydney NSW, Australia*

[2] *School of Chemistry, Australian Centre for Nanomedicine, University of New South Wales, Sydney NSW, Australia*

[3] *Adult Cancer Program, Lowy Cancer Research Centre, UNSW Sydney, Sydney, NSW 2052, Australia*

[4] *School of Materials Science and Engineering, University of New South Wales, Sydney NSW, Australia*

Corresponding author's email: pallavi.srivastava@student.unsw.edu.au

In-vivo embryogenesis demonstrates a flawless example of self-organization using a combination of diffusible morphogens and the physical effect of the endometrial constraint as guidance for germ layer differentiation. Modulating physical environmental factors has supported the growth and development of a pre-gastrulation mouse embryos in-vitro. Although information is available on how diffusible morphogens affect gene expression and signalling dynamics, the mechanistic role in inducing these events is poorly understood. Due to ethical limitations with human embryos, in-vitro models present a better platform to understand the contribution of mechanical stress towards the differentiation and self-organization capacity of pluripotent stem cells. Previous studies have demonstrated a role of geometry in germ layer organization of ESCs in response to BMP4 induction. Here, we present an in-vitro gastrulation model, which uses polyacrylamide hydrogels as culture substrates to present physical factors like stiffness and geometry to the cells creating a self-sufficient system for differentiation and spatial organization using iPSCs, without the need of using exogenous induction. We demonstrate that substrate stiffness and geometry control iPS cells differentiation into a SOX17+ endodermal and a T/Brachyury+ population, which represents primitive streak/mesoderm by fluorescence imaging and qPCR results.These colonies confine the cells causing cytoskeleton mediated nuclear shape alterations, inducing the signalling dynamics to create these gastrulation-like events, enhanced in a 3-D environment, implicating the crucial role of mechanics of the culture substrates. This model presents a simple approach to understand how iPSCs interpret their physical microenvironment to alter cell identity and spatial organization, which better represents the in-vivo environment.

Keywords

Gastrulation; Micropatterning; biomaterials

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Abstract **#1842**

LONG-TERM STABILIZATION OF THREE-DIMENSIONAL PERFUSABLE MICROVASCULAR NETWORKS IN MICROFLUIDIC DEVICES

HO-YING WAN$_P$[1], JACK Chun Hin CHEN[2], Qinru XIAO[2], Christy Wingtung WONG[1], Yi-Ping Megan HO[2], Roger D. KAMM[3], Sebastian BEYER[2], Anna Maria BLOCKI[1]

[1] *Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong*

[2] *Department of Biomedical Engineering, Faculty of Engineering, The Chinese University of Hong Kong*

[3] *Department of Biology and Mechanical Engineering, Massachusetts Institute of Technology*

Corresponding author's email: wanhoying@link.cuhk.edu.hk

The involvement of the microvasculature in many (patho-)physiological processes inspired the interest to engineer in vitro models that allow to dissect these complex biological processes with spatiotemporal resolution. Current state-of-the-art in vitro engineered human microvasculature is based on perfusable microvascular networks (MVNs) within microfluidic devices. Unfortunately, these MVN suffer from pruning and network retraction upon spontaneous vasculogenesis, leading to non-functional luminous structures.

To address this limitation, we developed cell culture methodologies by introducing a biophysical stimulus based on macromolecular crowding (MMC) into MVN cultures, composed of umbilical vein endothelial cells (HUVECs), forming the inner lining and mesenchymal stem cells (MSCs), taking up pericyte function. In both experimental groups MVNs formed within the first 2 days, exhibited patent lumina and were outlined with a basement membrane. Noteworthy, MVNs cultured without MMC regressed immediately after being formed while MMC led to perfusable MVN with a retention up to day 10. Quantification of major basement membrane components revealed an enrichment of collagen IV and laminin α5 in MMC supplemented MVN cultures. A functional contractility assay further confirmed a reduced contractility of MSCs and HUVECs in three-dimensional cultures.

Taken together, current data indicate that MMC stabilized MVN by promoting the formation of a robust basement membrane around microvessels and by toning down cell contractility that generally drives microvessel retraction. The here established platform integrates two key technologies based on MVNs in microfluidic devices and MMC and enables the modeling of various microvasculature-related (patho-) physiological processes with a close resemblance of physiological cellular composition.

Keywords

three-dimensional ; microvascular networks; microfluidic

Abstract **#1844**

ROS RELEASING HYDROGEL ENCAPSULATED WITH TONSIL-DERIVED MESENCHYMAL STEM CELLS ACCELERATE ITS REJUVENATION AND BONE FORMATION VIA G-PROTEIN TRANSLOCATION

Da Hyeon Choi[P][1], Kyeong Eun Lee[1], Inho Jo[2], Ki Dong Park[3], Yoon Shin Park[1]

[1] *Chungbuk National University*

[2] *Ewha Womans University*

[3] *Ajou University*

Corresponding author's email: dahyeon@chungbuk.ac.kr

A long-term serial cell expansion can induce stem cell senescence, which can cause a progressive decline in stem cell function and stemness, highlighting the need to identify specific biomarkers for senescent cells. Reactive oxygen species (ROS), known as one of factors responsible for senescence, rather requires low levels of ROS to maintain quiescence and self-renewal. Based on this, we exposed the tonsil-derived mesenchymal stem cells (TMSC) to a low level of ROS using sustained ROS releasing hydrogel and identified the new senescence biomarker and its effects on stem cell function as well as its expressional changes. Changes of transcriptomic and proteomic profiles of TMSCs were evaluated using control (5 – 8p) and culture-aged senescent TMSCs (20 – 25p). G-protein was identified as an MSCs specific hallmark of senescence. Changes of intracellular location of G-protein from cytosolic to the peri-nuclear region as well as significantly decreased cytosolic expression with replicative senescence. Next, cell surface G-protein expressing cells (CS-G-pro(+)) sorted by magnetic-activated cell sorting (MACS) method presented were applied for further experiment. The CS-G-pro(+) cell showed superior ability not only in differentiation ability but also in mitochondria metabolic efficacy and proliferation. In the calvarial defect rat model, bone regeneration efficacy was high when mixed with ROS releasing hydrogel. Taken, we suggest that the G-pro as an effective senescence biomarker of MSCs and ROS releasing hydrogel can control its regenerative potential as well as the proliferation of MSCs by regulation of the G-pro intracellular distribution.

Keywords

senescence; ROS; GRP78

Abstract **#1845**

ROLE OF ENDOSTATIN IN CHONDROGENIC DIFFERENTIATION IN NEONATAL MENISCAL CELLS

Valentina Herrera$_P$[1], Giuseppe Peretti[2,3], Paola Pocar[1], Laura Mangiavini[2,3], Silvia Modina[1], Alessia Di Giancamillo[1]

[1] *Department of Veterinary Medicine, University of Milan, Italy*

[2] *Department of Biomedical Sciences for Health, Università degli Studi di Milano, Italy*

[3] *IRCCS, Istituto Ortopedico Galeazzi, Milan, Italy*

Corresponding author's email: valentina.herrera@unimi.it

The success of cell-based approaches for the treatment of cartilage or fibro-cartilaginous tissue defects, requires an optimal cell source with chondrogenic differentiation ability that maintains its differentiated properties and stability following implantation. For this purpose, the aim of this study was to evaluate the use of endostatin (COL18A1), an anti-angiogenic factor, which is physiologically involved in meniscus development for cell differentiation. Neonatal porcine meniscal cells not yet subjected to mechanical stimuli were extracted, cultured in fibrin scaffolds, and treated for two different times with different concentrations of COL18A1. At the end of the treatments, the scaffolds were subjected to biochemical, molecular, and histochemical analysis. The results showed that high concentrations of COL18A1 increased the production of glycosaminoglycans (GAGs), without particular variations in the cellularity index (DNA content) compared to the untreated experimental group (UNT), resulting in an increase in the GAGs/DNA ratio. These data were supported by the molecular analysis of type II collagen (COL2A1, marker of cartilaginous-like tissue), SRY-Box Transcription Factor 9 (SOX9, early marker of chondrogenicity) and type I collagen (COL1A1, marker of fibrous-like tissue), where the expression of COL2A1 and SOX9 increased with increasing endostatin concentration and the expression of COL1A1 decreased compared to UNT group. Histological studies (Safranin-O staining) confirmed that high concentrations of endostatin were more effectives, laying the foundations for researches evaluating the involvement of this anti-angiogenic protein in the differentiation of avascular tissues.

Keywords

Endostatin; Scaffolds; Meniscal cells

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Abstract **#1846**

ANNEALED MICROGELS FOR OSTEOCHONDRAL LESIONS REGENERATION BY ENDOGENOUS BONE MARROW MESENCHYMAL STEM CELL MIGRATION

Anna Puiggali-Jou[1], Katharina Maniura-Weber[2], Marcy Zenobi-Wong[1]

[1] Tissue Engineering and Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zürich, Otto-Stern-Weg 7, Zürich 8093, Switzerland.

[2] Laboratory for Biointerfaces, Empa, Swiss Federal Laboratories for Materials Science and Technology, St. Gallen 9014, Switzerland.

Corresponding author's email: anna.puiggalijou@hest.ethz.ch

It is estimated that about 0.5% of the world's population will require an articular cartilage intervention at some point in their life. [1] Current treatment options for osteochondral (OC) lesions are only marginally successful, and if left untreated, lead to osteoarthritic joint disease, one of the major sources of disability world-wide. [2] Many of the common surgical approaches to treat cartilage lesions such as microfracture, are dependent on the efficient migration of cells from the underlying subchondral bone to ensure successful regeneration of the tissue. However, the extent of cell migration and hence the clinical outcome, is highly variable. [3] To overcome this critical limitation, it is necessary to study the chemical and physical properties that would induce bone marrow mesenchymal stem cell (BMSCs) migration in a 3D matrix. We propose a simple and fast method to produce macroporous annealed particle hydrogels by mechanical disruption of HAMA hydrogels [4] and secondary crosslinking them using a cell-friendly transglutaminase enzymatic reaction [5]. This design presents enough void space that can naturally facilitate the endogenous migration of BMSCs. Microgels with different diameters (Ø = 48 ± 17.5 µm and 12.8 ± 5.2 µm corresponding to a void space of 25% and 35 %, respectively) and different degree of substitution (10% and 50% corresponding to a compression modulus of 7.2 ± 0.4 and 20.4 ±1.7 KPa, respectively) were evaluated. We believe these advanced, yet biocompatible materials, provide an ideal 3D environment for promoting cell migration and differentiation leading to a fully functional OC repair.

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Abstract **#1848**

DELAYED THE ESRD PROGRESSION BY TRANSPLANTING 3D PRINTED OMENTAL PATCH

Hyunoo Jo[P][1], Boyun Kim[1], Jina Ryu[1]

[1] ROKIT HEALTHCARE, Inc

Corresponding author's email: whgusdn4@rokit.co.kr

Chronic kidney disease (CKD), caused by various causes, leads to the end-stage of renal diseases (ESRD). Dialysis and kidney transplant are the only treatments existed in the market for ESRD. Currently, there is no practical renal replacement therapies. We developed a new treatment using autologous omental tissue that can delay the progression of ESRD. Omentum is one layer of the peritoneum, known for a rich source of biological materials that enhance tissue growth and has rich matrix. Also, growth factors such as bFGF, PDGF, HGF, and VEGF in the omentum can play a key role in renal regeneration. We prepared the bio-ink using a micronizing omental tissue, then printed it in the form of a therapeutic patch. A 3D printed omental patch was applied to Kidney of CKD rat model. We transplanted the omental patch to a renal subcapsular layer in unilateral ureteral obstruction (UUO) rat, and kidneys were histologically analyzed after two weeks of transplantation. Reduced glomerulosclerosis and tubular injury were observed in the omental patch UUO group compared to the untreated UUO group. Also, high cellular proliferation was observed in regions near the patch. Next, we selected a 5/6 nephrectomy CKD model to determine the effect of omental patch on renal function. The untreated 5/6 nephrectomy group showed a higher BUN and Creatinine range, while the omental patch 5/6 nephrectomy group showed a normal range in BUN and Creatinine. Therefore, we confirmed that the omental patch delays ESRD progression by providing protective effects to kidney disease.

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Abstract **#1849**

3D BIOPRINTING OF HYALURONAN-TYRAMINE MICROGEL BIOINKS WITH TUNABLE POROSITY FOR AURICULAR CARTILAGE REGENERATION

Killian Flegeau[P][1], Marcy Zenobi-Wong[1]

[1] *Department of Health Science and Technology, ETH Zurich, Zurich, Switzerland*

Corresponding author's email: killian.flegeau@hest.ethz.ch

Microtia is a congenital deformity affecting 1 of every 7,000 births worldwide and causing psychological morbidity1. Autologous rib cartilage graft remains the “gold standard” but is associated with high co-morbidity and risk of failure. 3D bioprinting of tissue-engineered cartilage was proposed to recreate the complex auricular shape but suitable bioinks combining printability and supporting maturation to functional tissue remain scarce. Granular hydrogels, made of annealed microgels, are excellent candidates combining injectability and porosity2. In this study, we evaluated the use of sized microgels as bioinks with tunable porosity for auricular cartilage regeneration. Enzymatically crosslinked hyaluronan-tyramine (HA-Tyr) hydrogels were fragmented using grids with aperture of 40, 100 or 500 µm. Microgel bioinks mixed with 10 mio/mL auricular chondrocytes suspension were microextrusion printed (discs of 8x2 mm) with excellent viability (>75% at D1), self-healing and shear-recovery properties, and stabilized by secondary crosslinking of tyramine residues. Scaffolds were matured for 3 weeks in vitro and subcutaneously implanted for 6 weeks in nude mice. Decreasing microgels size resulted in increased porosity (from 8 to 21%) and initial stiffness (from 5.5 to 10 kPa) and favored cell invasion of acellular constructs in vivo. Cells uniformly spread between the microgels in 40 µm samples but clustered in larger 100 and 500 µm microgels, resulting in micro-pellet formation. Cartilage maturation was confirmed in vitro with stiffening of the gels (100 kPa), upregulation of key chondrocyte genes, and deposition of collagen II and glycosaminoglycans. Altogether, secondary crosslinked HA-Tyr microgels are promising bioinks for auricular cartilage regeneration.

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Abstract **#1850**

IMPROVED FIBER ALIGNMENTS VIA CONTROLLED FIBER DEPOSITION IN NEAR FIELD ELECTROSPINNING TECHNIQUE

Elahe Kazemi$_P$[1], Mansure Kazemi[2], Hasti Esmaeili[3], Mohammad Rafienia[1], Alireza Mohebbi[4], Liang Hao[5,6], Seyed Ali Poursamar[7]

[1] *Biosensor Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

[2] *Department of Applied Cell Sciences, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran*

[3] *Department of Biomedial Engineering, Faculty of Engineering, University of Isfahan, Isfahan, Iran*

[4] *Energy Division, Iran Polymer and Petrochemical Institute, P.O. Box 14965-115, Tehran, Iran*

[5] *Gemological Institute, China University of Geoscience, Wuhan 430074, People's Republic of China*

[6] *Advanced Manufacturing Research Institute, Hubei Gem and Jewelry Engineering Research Centre, Wuhan 430074, People's Republic of China*

[7] *Biomaterials, Nanotechnology, and Tissue Engineering Group, Advanced Medical Technology Department, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran*

Corresponding author's email: elahe.kazemi.ra@gmail.com

Near-field electrospinning (NFES), is a powerful technique for direct-writing of micro/nanofibers, with the potential of creating detailed structures. This ability opens an interesting perspective for the position-controlled deposition and precise integration of aligned individual fibers which greatly expands their application in various fields [1]. However, the utilization of these structures, is limited by the maximum achievable height of controlled fiber deposition, beyond which the structure becomes increasingly disordered [2]. The aim of this study was to investigate the impact of distance between adjacent fibers on the deposition of fibers and their alignment by increasing the constructed layers. For this purpose, a 2 ×2 cm network-like pattern by inter fiber distance in the range of 0.1 to 1 mm was designed and a network of polycaprolactone (PCL)-gelatin composite was fabricated up to 50 layers. The operational conditions were investigated and optimized as follow; voltage of 1200 v, the feed rate of 14 mm/s, nozzle-to-collector distance of 1 mm and flow rate of 10 µL/h. According to experimental data, the alignment index defined as actual distance relative to the theoretical pre-defined distance was approached to 1 by increasing the inter fiber distance from 0.3 to 1 mm. The random orientation of the printed fibers at distances less than 0.3 mm can be attributed to the charge build-up on the deposited polymer producing unwanted coulombic forces [3]. This study demonstrates the practical limits of inter-fiber distance and necessity of electrostatic control on the level of orientation that can be achieved via NFES method.

Keywords

Near Field Electrospinning Technique; Cancer; Cartilage

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Abstract **#1851**

DISSECTING THE EFFECT OF A 3D MICRO-SCAFFOLD ON THE TRANSCRIPTOME OF NEURAL STEM CELLS WITH COMPUTATIONAL APPROACHES: FOCUS ON MECHANOTRANSDUCTION

Letizia Messa[P][1,2], Bianca Barzaghini[1], Federica Rey[3], Cecilia Pandini[2], Gian Vincenzo Zuccotti[3], Cristina Cereda[2], Stephana Carelli[3], Manuela Teresa Raimondi[1]

[1] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy*

[2] *Genomic and post-Genomic Center, IRCCS Mondino Foundation, Pavia, Italy*

[3] *Pediatric Research Center "Romeo ed Enrica Invernizzi", University of Milano, Milan, Italy*

Corresponding author's email: letizia.messa@mail.polimi.it

3D scaffolds are becoming more and more important in the field of regenerative medicine due to their ability to mimic the physiological microenvironment. In this field, Raimondi and colleagues applied two-photon polymerization (2PP) to fabricate the "Nichoid", able to maintain and stimulate the stemless of cells[1, 2]. To investigate the Nichoid's influence on cellular response, murine neural stem cells (NSCs) where expanded inside the micro-scaffold [2] and analysed through a whole transcriptomic approach [3]. Nichoids were fabricated by 2PP. 10000 NSCs were expanded inside the engineered niches and in control conditions for 7 days and then analysed through RNA-Seq. We demonstrate that the Nichoid impacts the biological and genetic response of stem cells, as genes strongly connected to mechanobiological functions emerged. We fully dissected this mechanism highlighting how the changes start at a membrane level, with subsequent alterations in the cytoskeleton, signaling pathways, and metabolism, all leading to a final global modification of gene expression. Our findings highlight the Nichoid's ability to induce changes in cultured stem cells at any molecular level without any other chemical agents, allowing recreating the stem cells adhesion, proliferation and cell signaling that best mimics physiological conditions. We highlight the Nichoid's importance for regenerative medicine approaches based on the development of stem cell therapies.

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Abstract **#1852**

ENZYMATICALLY CROSSLINKED ZWITTERIONIC MICROGELS FOR BIOPRINTING OF MACROPOROUS SCAFFOLDS

Maryam Asadikorayem$_P$[1], František Surman[1], Patrick Weber[1], Marcy Zenobi-Wong[1]
[1] *Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zürich*
Corresponding author's email: maryam.asadikorayem@hest.ethz.ch

Zwitterionic hydrogels have high potential for various biomedical applications, due to their biocompatibility and low biofouling properties [1, 2]. However, the current methods used for preparation of zwitterionic hydrogels are limited and their potential in innovative biofabrication methods such as bioprinting has not been explored yet. Here, we present a novel and scalable strategy to make zwitterionic microgels that are printable and can be enzymatically crosslinked to make stable, macroporous constructs. Bulk hydrogels are produced via photopolymerization of zwitterionic carboxybetaine acrylamide monomer using gelatin methacryloyl as crosslinker and tyramine acrylamide as functional comonomer to allow for secondary crosslinking via horseradish peroxidase and H2O2. To introduce printability as well as macroporosity of the final construct, the bulk hydrogel is mechanically fragmented into microgels of 100–40 µm in size. This microgel bioink showed optimal shear thinning and shear recovery properties required for extrusion printing with high shape fidelity. Enzymatic crosslinking of microgels resulted in a macroporous structure, optimal for facilitated diffusion and cell-cell interaction [3, 4]. In vitro experiments with human chondrocytes showed excellent biocompatibility with more than 90% viability and enhanced tissue maturation. Moreover, cells were encapsulated in the pores between microgels and bioprinted with retained high cell viability. Bioprinting of cell-laden microgel bioink was demonstrated by creating a 3D ear model which after enzymatic cross-linking, resulted in a mechanically stable construct in culture media. This versatile and highly biocompatible platform material shows great promise for bioprinting of diverse zwitterionic monomers which can be used for multiple tissue engineering applications.

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Abstract **#1853**

BIPHASIC BIOREACTOR FOR OSTEOCHONDRAL DRUG SCREENING AND TOXICITY ASSESSMENTS

Francesca Donnaloja[P][1], Giulio De Riccardis[1,2], Derek Nichols[3,4], Martina Avolio[1,2], Manuela Teresa Raimondi[1], Riccardo Gottardi[2,5,6]

[1] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy*

[2] *Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA*

[3] *Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA*

[4] *Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA*

[5] *Fondazione Ri.MED, Palermo, PA*

[6] *Division of Pulmonary Medicine, Department of Pediatrics, University of Pennsylvania, Philadelphia, PA*

Corresponding author's email: francesca.donnaloja@polimi.it

The increasing prevalence of osteoarthritis and the modest efficacy of available treatments represent an incentive to develop new therapeutic strategies. However, the lengthy and increasingly expensive process of pharmaceutical innovation and the significant percentage of failed pre-clinical trials demonstrate an urgent need for realistic tools for in vitro drug screening. In this context, starting from previously optimized bioreactor [1], we developed a new millifluidic bioreactor able to accommodate biphasic cell construct perfused by two separate culture media in order to reproduce the crosstalk between articular cartilage and subchondral bone, while providing an accurate assessment of the mechanism of action and potential drug toxicity. The implemented CFD analysis and biological tests validated the designed biphasic bioreactor capability in guaranteeing cell viability, continuous optical monitoring, no mixing of fluids in the culture chamber hosting each tissue phase. Moreover, we validated the designed device in inducing two specific differentiation pathways in the same chamber. These results confirmed the new biphasic bioreactor as a suitable device for multiphasic tissue culture, where optical accessibility for real-time monitoring at the tissue interface is a fundamental requirement to capture potential interactions of candidate treatments in drug screening and toxicity assessment. Once scaled for high throughput, our biphasic bioreactor will be an essential step in developing new strategies to prevent and treat joint diseases.

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Abstract **#1854**

POROUS BIODEGRADABLE MICROCARRIERS FOR IPSC-DERIVED CARDIOMYOCYTE EXPANSION & DIFFERENTIATION

Annalisa Bettini$_P$[1], Daniel Stuckey[2], Richard Day[1]

[1] *Centre for Precision Healthcare, UCL Division of Medicine, University College London, London, WC1E 6BT, UK*

[2] *Centre for Advanced Biomedical Imaging, UCL Division of Medicine, University College London, London, WC1E 6DD, UK*

Corresponding author's email: annalisa.bettini.17@ucl.ac.uk

Cell therapy is a potential novel treatment for cardiac regeneration. Numerous studies have attempted to transplant cells to regenerate myocardium lost during myocardial infarction. To date, only minimal improvements to cardiac function have been reported in clinical trials. This is likely to arise from low cell retention following delivery and high cell death after transplantation.

The current study aims to improve intramyocardial delivery of viable cells by using an injectable biodegradable substrate that supports cell attachment and growth of cardiomyocytes derived from induced pluripotent stem cells (iPSC).

Highly porous microcarriers <250 µm were fabricated from 2% (w/v) 75:25 poly(DL-lactide-co-glycolide) using Thermally Induced Phase Separation (TIPS). Pre-incubating the microcarriers with recombinant human vitronectin (VTN-N; 0.5 µg/ml) resulted in an increase in iPSC attachment (93.4% vs 2.9% control; attached cells as % of seeded cells, n=5, $P \leq 0.0001$). The attached cells proliferated (doubling time≤24 hrs, n=3). iPSC attached to the microcarriers retained their pluripotent phenotype, demonstrated by positive expression of markers, SOX2, OCT4, TRA-1-60, and SSEA4. Pluripotency was further demonstrated by differentiation of TIPS-iPSC into a mixed cardiomyocyte-like population exhibiting a beating phenotype for up to 40 days in culture.

This work demonstrates that TIPS microcarriers offer a supporting matrix for culturing and differentiating iPSC and may provide an injectable biomaterial for cardiac regeneration.

Keywords

enhancing cardiac regeneration; stem cell derived cardiomyocytes; porous biodegradable microcarriers

Abstract **#1855**

CT-VISIBLE MICROSPHERES ENABLES IN VIVO TRACKING OF BIOMATERIAL DISTRIBUTION AFTER ULTRASOUND-GUIDED INTRAMYOCARDIAL INJECTION

Annalisa Bettini[P][1], P. Stephen Patrick[2], Richard Day[1], Daniel Stuckey[2]

[1] *Centre for Precision Healthcare, UCL Division of Medicine, University College London, London, WC1E 6BT, UK*

[2] *Centre for Advanced Biomedical Imaging, UCL Division of Medicine, University College London, London, WC1E 6DD, UK*

Corresponding author's email: annalisa.bettini.17@ucl.ac.uk

Efficacy of cell therapies are often diminished by low cell retention. A method to visualise the location of grafted cells and biomaterials after delivery could be used to optimise therapies by verifying injection success, retention of the therapeutic and distribution of the product. To address these challenges an injectable cell-substrate consisting of highly porous microspheres containing the computed tomography (CT) contrast agent barium sulphate (BaSO4) was developed.

Porous microspheres (<250 µm) were fabricated via Thermally Induced Phase Separation (TIPS) using a 2% (w/v) 75:25 poly(DL-lactide-co-glycolide) polymer solution containing a 20% (w/v) colloidal suspension of particulate BaSO4. Culture media conditioned with BaSO4-loaded microspheres (46.87 mg/ml) showed no significant toxicity when cultured with L929 fibroblasts for up to 7 days (maximum toxicity 12% vs 5% matched control media, n=4, $p<0.01$).

Suspensions of BaSO4-loaded microspheres generated high CT contrast in vitro (2023.6 Hounsfield Units) and were then tested in vivo. Three 50 µl injections of BaSO4-loaded microspheres (46.87 mg/ml in 60% GRANUGEL®; 21G needle) were delivered into the myocardium of Sprague Dawley rats under real-time ultrasound-guidance (n=6). Whole body CT scans revealed BaSO4-loaded microspheres at the injection site within the myocardium at 1 hour, 2, 4 and 6 days after injection. A low level of microspheres were visualised in off-target organs including lung, brain and liver (confirmed by histology), indicating some material was lost during injection.

These findings suggest that BaSO4-loaded microspheres can be used as a novel tool for optimising delivery techniques and tracking persistence and distribution of implanted products.

Keywords

trackable injectable therapies; imaging biomaterials; regenerative therapies

Abstract **#1856**

RETINOL-CROSSLINKED SUPRAMOLECULAR HYDROGELS FOR TISSUE REGENERATION AND ANTIOXIDANT DELIVERY

Antonio J Feliciano$_P$[1], Matthew B Baker[1], Lorenzo Moroni[1]

[1] *Maastricht University*

Corresponding author's email: a.feliciano@maastrichtuniversity.nl

Bioengineered corneas have been fabricated using a variety of synthetic or natural materials to transplant corneal keratocytes.1 However, control of keratocyte phenotype remains a challenge. If a regenerative construct activates a fibroblast-like phenotype then unfavorable ECM akin to scar tissue is produced which will adversely affect transparency and ultimately impair vision. All trans-retinoic acid (RA), as a supplement, has been shown to promote favorable non-scarring phenotype and keratocyte proliferation.2 We hypothesize that supramolecular materials formed via host-guest inclusion of retinol and cyclodextrin can recapitulate dynamics of the ECM but also act as an injectable drug-delivery scaffold. Herein, we confirm the complexation between retinol and ?-cyclodextrin (?CD) and proceed to use this interaction to build a supramolecular system with ?CD-functionalized alginate. We present host-guest complex characterization, synthesis of functionalized alginate, tunable stiffness of hydrogel, and cumulative release of retinol. In addition, we show supramolecular hydrogel behavior for example self-healing and shear thinning character from this interaction through rheological tests. A supramolecular environment built with bioactive molecules like this could facilitate corneal keratocyte proliferation but also be used for other tissue applications where tissue remodeling and tissue integration could be enhanced with antioxidant delivery.

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Abstract **#1857**

»JOINTPROMISE«: CONCEPT OF A MULTI-STEP, AUTOMATED PLATFORM FOR PRECISION MANUFACTURING OF JOINT IMPLANTS

Judith Krieger[P][1], Stan Snowball[2], Frank P. Luyten[2], Ioannis Papantoniou[2,3], Robert H. Schmitt[1,4]

[1] *Fraunhofer Institute for Production Technology IPT, Aachen, Germany*

[2] *Katholieke Universiteit Leuven, Belgium*

[3] *Foundation of Research and Technology Hellas, Greece*

[4] *Laboratory for Machine Tools and Production Engineering (WZL), RWTH Aachen, Germany*

Corresponding author's email: judith.krieger@ipt.fraunhofer.de

Osteoarthritis (OA) is the most frequently encountered chronic joint disease worldwide affecting more than 25% of the adult population. Joint injury, as a result of trauma, inflammation or infec-tion, leads to superficial and deep joint surface defects, which are risk factors in the development and progression of OA. Engineering precise osteochondral/joint tissues for the regeneration of such defects could mitigate this clinical and societal challenge. However, engineering suitable im-plants remains a challenge due to the complex structural organization and functionality of native joint tissue.
»JOINTPROMISE« aims to develop an automated process pipeline for precision manu-facturing of functional 3D living implants of the required structural complexity, paving the way for the creation of a tissue-engineered prosthesis to treat patients suffering from end-stage osteoar-thritis. The »JOINTPROMISE« platform will additionally boost productivity by transforming existing laboratory-scale Tissue Engineering processes into robust, large-scale and cost-effective production methods.
We elaborated the concept of a multistep automated platform for manufacturing high-complexity Joint-ATMPs by the production of progenitor cell aggregates and their subsequent maturation into microtissues, functioning as building blocks for the assembly of joint implants by multi-step 3D bio-printing. This concept will be implemented by building up an end-to-end automated pro-duction pipeline for Joint-ATMPs. After proving feasibility, safety and indications of clinical efficacy in large animal models, our Joint-ATMPs open up new possibilities in treatment of deep joint sur-faces to building a biological prosthesis for end-stage OA.
This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 874837.

Abstract **#1858**

4D BIOPRINTING WITH AUTOLOGOUS ADIPOSE TISSUE DERIVED ECM BIOINK ENABLES COMPLETE RE-EPITHELIALIZATIOIN OF CHRONIC DIABETIC FOOT ULCER WOUND WITHIN 4 WEEKS

Jeehee Kim$_P$[1]

[1] *ROKIT HEALTHCARE, Inc*

Corresponding author's email: Jeehee.kim@rokit.co.kr

The medical industry has been globally exploding together with innovative drugs and medical devices development. The latest biological research accelerates to discover new approaches of cell and gene therapy for repairing damaged human body. The fourth industrial revolution combined life, engineering and digital science strategically disrupts the traditional pharmaceutical medicine market to open a new era in healthcare business. Indeed, advances in three-dimensional (3D) bioprinting technology and their various applications in tissue engineering provide emerging solutions of precision medicine and customized healthcare. Since 3D bioprinting fabrication is the best way to recapitulate complex and functional human tissue and organ, we have developed the therapeutic 3D bioprinter called INVIVO to use in skin, cartilage, and cardiac regeneration. To find advanced treatment method particularly for wound healing, we tested our own 3D bioprinting system with autologous fat tissue for diabetic foot ulcer (DFU) treatment. The extracellular matrix (ECM) derived from nano-fat consisting of supportive proteins, growth factors and cytokines, has been reported as an important player to provide efficient 3D environments during cell proliferation and differentiation. In the clinical study, we printed minimally manipulated autologous ECM (MA-ECM) with bio-inks to apply onto the chronic wound site. Most of DFU patients showed significant reduction of wound size with distinct epithelization process only after a week treatment of 3D bioprinting MA-ECM. Therefore, we suggest 3D bioprinting system with MA-ECM as an alternative method for dressing materials that successfully promote the mechanism of skin reconstruction for DFU treatment.

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Abstract **#1859**

INTRACELLULAR LABEL-FREE DETECTION OF MESENCHYMAL STEM CELL METABOLISM WITHIN A PERIVASCULAR NICHE-ON-A-CHIP

Simone Perottoni[P][1], Nuno G Neto[2,3], Cesare di Nitto[4], Ruslan Dmitriev[5], Manuela T Raimondi[4], Michael G Monaghan[2,3,6,7]

[1] .Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, piazza Leonardo da Vinci, 32 – 20133 Milan

[2] Department of Mechanical, Manufacturing and Biomedical Engineering, Trinity College Dublin, Dublin 2, Ireland.

[3] Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse Street, Dublin 2, Ireland.

[4] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, piazza Leonardo da Vinci, 32 – 20133 Milan,

[5] .Tissue Engineering and Biomaterials Group, Department of Human Structure and Repair, Faculty of Medicine and Health Sciences, Ghent University, Ghent 9000, Belgium.

[6] Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland

[7] CÚRAM, Centre for Research in Medical Devices, National University of Ireland, Galway, Newcastle Road, H91 W2TY Galway, Ireland

Corresponding author's email: monaghmi@tcd.ie

Mesenchymal stem cells (MSCs) , experience influential microenvironmental conditions, which induce specific metabolic profiles that affect processes of cell differentiation and dysregulation of the immunomodulatory function. Reports focusing specifically on the metabolic status of MSCs under the effect of pathophysiological stimuli - in terms of flow velocities, shear stresses or oxygen tension – do not model heterogeneous gradients, highlighting the need for more advanced models reproducing the metabolic niche. Organ-on-a-chip technology offers the most advanced tools for stem cell niche modelling thus allowing for controlled dynamic culture conditions while profiling tuneable oxygen tension gradients. However, current systems for live cell detection of metabolic activity inside microfluidic devices require the integration of microsensors. The presence of such microsensors pose the potential to alter microfluidics and their resolution does not enable intracellular measurements but rather a global representation concerning cellular metabolism. Here, we present a metabolic toolbox coupling a miniaturised in vitro system for human-MSCs dynamic culture, which mimics microenvironmental conditions of the perivascular niche, with high-resolution imaging of cell metabolism. Using Fluorescence Lifetime Imaging Microscopy (FLIM) we monitor the spatial metabolic machinery and correlate it with experimentally validated intracellular oxygen concentration after designing the oxygen tension decay along the fluidic chamber by in silico models prediction. Our platform allows for the metabolic regulation of a metabolic profile to MSCs, mimicking the physiological niche in space and time, and its real-time monitoring representing a functional tool for modelling perivascular niches, relevant diseases and metabolic-related uptake of pharmaceuticals.

Abstract **#1860**

HUMAN SALIVARY GLAND RESIDENT EPITHELIOMESENCHYMAL STEM CELL DERIVED EXTRACELLULAR MATRIX (ECM) IMPROVED ORGANOID FORMATION AND MATURATION

Yeo-Jun Yoon[P][1], Joshua Jaeyun Kim[2]

[1] *Yonsei University College of Medicine*

[2] *ROKIT HEALTHCARE, Inc*

Corresponding author's email: joshua@rokit.co.kr

Salivary gland organoids have therapeutic potential for treating salivary gland hypofunction. We developed salivary gland organoid, in which bone marrow stem cell (BMSC)-derived extracellular matrix (ECM) supports formation of salivary gland organoids and growth of acinar cells. We tested the regenerative potential of salivary gland organoids after transplantation into SCID mice model for Sjögren syndrome. Compared to the Matrigel conditioned media group, organoid grown with human primary BMSC derived ECM group was not only equivalent in growth rate, but also superior in ductal cell marker K5/K7 expression level at the basal/ luminal position of the organoid. Furthermore, AQP5, a pro-acinar marker, was identified, and Amy1 was present in the cytosol. In Amy1 measurement, Amy1 activity per unit cell was higher than that of Matrigel group. Furthermore, in the evaluation of other salivary gland functional markers such as BHLHA15, MUC7, KRT7, ACTA2 and CCND1, salivary gland organoid grown with BMSC ECM showed 2 folds or more functional enhancement compared to Matrigel group. Next, we implanted fully matured salivary gland organoid grown with BMSC ECM into SCID mice. Salivary gland organoid transplants showed salivary gland architectures at 2 weeks after implantation and contributed structural and functional rescue of salivary gland dysfunction in SCID mice. In conclusion, salivary gland organoid using primary human parotid gland grown with BMSC ECM successfully recapitulated the specific characteristics of human salivary gland. We expect this salivary gland organoid offers opportunities not just as a practical research tool but as a clinically translatable solution for medical treatment.

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Abstract **#1862**

WHOLE HUMAN EXTRACELLULARMATRIX (ECM) DERIVED FROM NEONATAL DERMAL FIBROBLAST CULTURE ACHIEVED COMPLETE BURN WOUND REGENERATION WITHOUT FORMING SCAR

Joshua Jaeyun Kim$_P$[1]

[1] *ROKIT HEALTHCARE, Inc*

Corresponding author's email: joshua@rokit.co.kr

WHO reports over 11 million people suffer from sever burn wound not only in traumatized body condition but also in post injury psychological trauma. Currently, there is no one time treatment that prevent further infection and promotes scarless skin regeneration at the same time. We derived human whole dermal ECM from neonatal dermal fibroblast culture and prepared in lyophilized powder form for burn wound treatment. In porcine model, 4cm x 4cm burn wounds (between 2nd and 3rd degree burn) were created. ECM powder dissolved in saline was sprayed directly on the derbidemented wounds. Re-epithelialization was achieved in 14 days and 35 days follow up showed fast skin regeneration pattern without contraction compared to control group (no treatment). At day 35, ECM treated group showed complete re-epithelialization with hair without scar while control group showed scar formation due to contraction. Furthermore, proteomic analysis showed ECM applied group had minimal amount of collagen type I content similar to that of normal undamaged skin. In histological analysis, rich collagen type III, which is main collagen type in dermis, was found in newly formed dermis of ECM treated group. However, collagen type I was main collagen type in control group. We also confirmed the reformed basedment membrane. Moreover, in immunohistological analysis, CTGF (connective tissue growth factor associated with fibrotic tissue formation) level was 4 folds less than scar formed control group. We confirmed that whole human ECM from matching tissue type cell provided a regeneration niche that is a minimal requirement for dermal regeneration.

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Abstract **#1863**

CHITOSAN-NANOCRYSTALLINE CELLULOSE SCAFFOLDS FOR BONE TISSUE ENGINEERING: EFFECT OF TWO DIFFERENT CROSSLINKING AGENTS ON THE PHYSICOCHEMICAL PROPERTIES OF THE SCAFFOLDS

Fatemeh Doustdar$_P$[1], Ali Olad[1]

[1] *University of Tabriz*

Corresponding author's email: f.doustdar@tabrizu.ac.ir

In the current study, the porous scaffolds based on chitosan and nanocrystalline cellulose polymers were fabricated for bone tissue engineering through the freeze-drying method. The effect of calcium cation as an ionic crosslinking agent and glutaraldehyde as a covalent crosslinking agent on the different features of the scaffolds was investigated. The accuracy of the syntheses was approved using the FTIR technique. The morphological and mechanical properties of scaffolds were evaluated through SEM imaging and tensile tests, respectively. Moreover, the water uptake capability of the scaffolds was investigated in PBS buffer (pH=7.4). The SEM images showed that the scaffolds are highly porous which provides proper surroundings for cell growth, cell migration, and transmission of nutrients and waste to or from cells. In addition, the scaffold which crosslinked by glutaraldehyde possesses a more uniform structure with bigger pores compared with the scaffold crosslinked by the calcium cation. Moreover, tensile tests represent that Young's modulus of glutaraldehyde-crosslinked scaffold is 2.5 folds greater than that of the ion-crosslinked scaffold. It can be concluded that the glutaraldehyde-crosslinked scaffold has better mechanical properties and a more stable 3D network. Furthermore, the water absorption capacity of the glutaraldehyde-crosslinked scaffold was higher than that of the ion-crosslinked scaffold. This can be attributed to the stable structure of the glutaraldehyde-crosslinked scaffold which can absorb and retain greater amounts of water. In this research, the glutaraldehyde-crosslinked scaffold showed better physicochemical properties than the calcium cation-crosslinked scaffold.

Keywords

Chitosan; Nanocrystalline cellulose ; Scaffold

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Abstract **#1865**

TWO-PHOTON POLYMERIZATION OF SYNTHETIC SCAFFOLDS FOR MICROGRAVITY APPLICATIONS BY MEANS ON DEFORMABLE SUBSTRATE

Alessandra Nardini[P][1], Claudio Conci[1], Emanuela Jacchetti[1], Giulio Cerullo[2], Rebeca Martínez Vázquez[2], Roberto Osellame[2], Gianni Ciofani[3], Manuela Teresa Raimondi[1]

[1] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy*

[2] *Istituto di Fotonica e Nanotecnologie (IFN)-CNR) and Department of Physics, Politecnico di Milano, Milano, Italy*

[3] *Istituto Italiano di Tecnologia (IIT), Smart Bio-Interfaces, Pontedera (PI), Italy*

Corresponding author's email: alessandra.nardini@polimi.it

Recently, tumor research focused onto microgravity cell cultures, analyzing the epigenetic effects in malignancy cellular behavior [1]. Nowadays, no dynamic culture system in use with microgravity conditions showed the capability to generate truly in vitro 3D tissue models by having a controlled geometry at the cellular scale [2]. Those systems, engineered to reach orbiting laboratories, as into the International Space Station, are limited to non-frangible substrates due to regulations. Therefore, we developed high resolved (~100 nm) synthetic 3D cell culture scaffolds [3] suitable to microgravity and zero-gravity applications. They are fabricated on a commercial non-frangible cyclic-olefyn-copolymer by the two-photon polymerization (2PP) into a biocompatible photoresist [4, 5].

Given the gap between mechanical properties, the 3D model stability under the stress conditions involved by the flexibility of the substrate, was computationally evaluated first. Then, we developed a brand-new protocol for substrate preparation and polymerization.

This study proved the feasibility of the 2PP microfabrication of biocompatible scaffolds on deformable non-frangible substrates, thus representing a great promise in 3D cell culture substrates production for microgravity and zero-gravity applications.

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Abstract **#1866**

EXTRACELLULAR MATRIX CHANGES IN GLAUCOMATOUS TRABECULAR MESHWORK EFFECT THE RATE OF ADULT STEM CELL DIFFERENTIATION

Olivia Kingston[P][1], Carl Sheridan[1], Rachel Oldershaw[1], Annette Meeson[2], Don Wellings[3]

[1] *Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, L7 8TX, UK*

[2] *Institute of Genetic Medicine, University of Newcastle, International Centre for Life, Newcastle, NE1 3BZ, UK*

[3] *Spheritech Ltd., The Heath Business and Technical park, Runcorn, WA7 4Qx, UK.*

Corresponding author's email: Olivia.kingston@liverpool.ac.uk

The trabecular meshwork (TM) is an essential tissue in the eye that facilitates aqueous humour outflow and filtration. In glaucoma patients, TM cellularity decreases, and extracellular matrix (ECM) accumulates, disrupting aqueous humour outflow and causing a rise in intraocular pressure. This damages optic nerves and leads to irreversible blindness(1). Adult stem cells in the TM (TMSCs) may offer a solution this problem, as they proliferate in response to damage caused by laser trabeculoplasty(2). We investigate the possibility for TMSCs to differentiate on ECM deposited by glaucomatous TM cells and therefore the potential to repopulate the TM in glaucoma patients.

Primary TM cells were cultured from dissected donor TM tissue. TMSCs were isolated over 7 days by sphere culture, a method encouraging growth of stem cells whilst initiating apoptosis of differentiated cells. Spheres were differentiated on ECM deposited by glaucomatous (GTM3) and normal (NTM5) immortalised TM cell lines for 7 days.

TMSC spheres successfully attached to tissue culture plastic (TCP) as well as the GTM3 and NTM5 ECM coating within 24 hours. Irrespective of ECM coating, spheres differentiated into cells with TM cell morphology. However, after 5 days, cells on GTM3 ECM had similar morphology to that of healthy primary TM cells and spheres were absent, whereas structures resembling spheres remained on TCP and NTM5 ECM coated wells.

TMSCs appear to differentiate at different rates on ECM from GTM3 compared to NTM5. Further studies will compare TM cell markers with primary cells; as well as assess TM phagocytic function.

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Abstract **#1867**

BIOPRINTING SPATIALLY ORGANISED CARTILAGE BY LEVERAGING CELLULAR SELF-ORGANISATION

Ross Burdis[P][1,2], Daniel J. Kelly[1,2,3,4]

[1] *Trinity Centre for Biomedical Engineering, Trinity College Dublin, Ireland*

[2] *Department of Mechanical, Manufacturing & Biomedical Engineering, Trinity College Dublin, Ireland*

[3] *Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland*

[4] *Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland*

Corresponding author's email: burdisr@tcd.ie

Tissue engineering strategies leveraging cellular self-organisation (SO) are capable of producing hyaline-like cartilage grafts [1,2], although recapitulating the spatial organisation of the tissue's collagen network remains a challenge. Additionally, current methods to securely fix such engineered tissues within joint defects are inadequate, leading to graft loosening and implant failure [3]. This study seeks to address both challenges by developing a 3D printed device capable of firstly guiding the SO of jetted MSCs to generate a structured engineered cartilage, and secondly, securely fixing such grafts into the subchondral bone. First, a monolithic polycaprolactone implant composed of an overlying microwell array (designed to direct engineered tissue growth) and an underlying anchoring pin was fabricated by fused deposition modelling. Biofabrication of the cartilage graft involved the automated deposition of MSCs into microwell arrays of the implant via inkjet bioprinting. Inkjetting enabled the accurate deposition of high cell numbers (up-to 264 x 106 cells/mL) in low media volumes (< 1 µL) into individual microwells. Within these microwells, in the absence of any adhesive sites, cells self-organised and in chondrogenic culture conditions formed a thick and biomimetic cartilage. The engineered tissue was rich in cartilage matrix-specific extracellular matrix (ECM) components and importantly exhibited a 'Benninghoff' collagen architecture. Finally, we demonstrated that in dynamic culture conditions, using a custom bioreactor, the quality of the engineered cartilage could be enhanced (to near native levels of cartilage ECM components), forming a thick (>1 mm thick) spatially organised tissue.

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Abstract **#1868**

BIOPRINTED CANCER MODELS: CHARACTERIZATION OF MATRIGEL AND ALGINATE-GELATIN-MATRIGEL BIOINKS

Paola De Stefano$_P$[1], Elena Bianchi[1], Francesco Briatico-Vangosa[2], Gabriele Angelo Dubini[1]

[1] *Laboratory of Biological Structure Mechanics (LaBS), Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy*

[2] *Polymer Engineering Group, Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy*

Corresponding author's email: paola.destefano@polimi.it

Cancer is the second leading cause of death worldwide. Despite the huge efforts to find effective treatments, failure rate is still high [1]. Bioprinted cancer models represent a significant improvement for drug discovery by mimicking the complex physiological 3D architecture [2]. Matrigel is the most common basement membrane used in vitro as substrate for 3D cell culture, as it is very similar to the physiological one [3]. However, its use is challenging due to its physico-chemical properties. Therefore, it is often used in combination with other polymers. To obtain the desired constructs, extensive knowledge of materials properties is strictly necessary.

To retrieve these characteristics, we performed rheological measurements in order to estimate the dynamic viscosity. Considering the wide range of possible shear rates acting on fluids, we started measuring viscosity at shear rates ranging from 0.1 to 100 [1/s]. Moreover, we also investigated the mechanical behaviour of hydrogels, evaluating their storage and loss moduli (G' and G'' respectively). All measurements were performed at 4, 20 and 37 °C. Preliminary results showed high variability in matrigel samples due to batch differences. This aspect significantly impairs filament extrudability and bioprinted fiber homogeneity. To overcome this limitation, matrigel dilutions with PBS and combinations with alginate/gelatin blend (4.5%/6.5% w/v in PBS) at different ratios increased reproducibility and improved bioprinting process, but impaired scaffolds mechanical stability. Therefore, we will evaluate swelling/degradation properties within three weeks to further characterize hydrogels capability to retain their structures over short and long time period cultures.

Keywords

bioprinting; matrigel characterization; in vitro cancer model

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Abstract **#1869**

DEVELOPMENTALLY INSPIRED MICROTISSUES AS BUILDING BLOCKS FOR THE BIOFABRICATION OF OSTEOCHONDRAL GRAFTS FOR BIOLOGICAL JOINT RESURFACING

Ross Burdis[P][1,2], Farhad Chariyev-Prinz[1,2], David Browe[1,2,3], Kian F. Eichholz[1,2,3], Bin Wang[1,2], Daniel J. Kelly[1,2,4,3]

[1] *Trinity Centre for Biomedical Engineering, Trinity College Dublin, Ireland*

[2] *Department of Mechanical, Manufacturing & Biomedical Engineering, Trinity College Dublin, Ireland*

[3] *Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland and Trinity College Dublin, Dublin, Ireland.*

[4] *Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland.*

Corresponding author's email: burdisr@tcd.ie

Autologous osteochondral transplantation (AOT) has been shown to outperform other clinical joint repair strategies[1,2], and is the only treatment that consistently resurfaces the joint with a hyaline cartilage tissue. However, several shortcomings limit AOTs universal uptake[3]. This project sought to use two phenotypically distinct populations of cartilage microtissues as biological 'building blocks' to fabricate biomimetic osteochondral plugs capable of regenerating critically sized synovial joint defects. The chondral region of the implant was engineered using stable cartilage microtissues that were combined, allowed to fuse, and self-organize (SO) into a homogenous hyaline-like cartilage tissue. Hypertrophic cartilage microtissues, primed to recapitulate the developmental process of endochondral ossification, were used within the osseous region of the implant. The engineered implant was evaluated in a pre-clinical, large animal (caprine) model. A novel method for forming phenotypically distinct cartilage microtissues, in a medium/high-throughput fashion was developed. The hypertrophic cartilage microtissues were rich in sGAGs and mineral, while the hyaline-cartilage tissues were rich in type II collagen with little evidence of hypertrophy. These distinct microtissues were spatially localised into chondral and osseous phases of a 3D-printed polymer implant that further functioned to guide the SO process, supporting the generation of a zonally defined collagen network in the chondral layer. After 6 months in vivo bone ingrowth into the osseous region was observed, stabilizing the implant, and facilitating the chondral region of the implant to support the restoration a near-normal articular surface. The cartilage repair tissue was superior to empty control defects, where little spontaneous regeneration was observed.

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Abstract **#1870**

DEVELOPING A HUMAN 3D IN VITRO MODEL OF OSTEOCHONDRAL DEFECT: OVERVIEW, POTENTIAL, OUTLOOK

Melania Maglio[P][1], Matilde Tschon[1], Leonardo Vivarelli[2], Marco Govoni[2], Martina Rocchi[2], Alessandro Mazzotta[2], Enrico Pennello[2], Carmelo Quinto[2], Stefania Pagani[1], Dante Dallari[2], Milena Fini[1]

[1] *IRCCS Istituto Ortopedico Rizzoli, Complex Structure Surgical Sciences and Technologies, Bologna, Italy*

[2] *IRCCS Istituto Ortopedico Rizzoli, Reconstructive Orthopaedic Surgery and Innovative Techniques - Musculoskeletal Tissue Bank, Bologna, Italy*

Corresponding author's email: melania.maglio@ior.it

The set- up of advanced in vitro model of bone and cartilage defects and fractures is a topic of growing interest for the evaluation of regenerative processes and innovative therapeutic solutions. However, despite the increasingly availability of biomaterials and therapies, there is still a relevant blank in the preclinical evaluation models. Few tissue models of osteochondral defects are described in literature, using mainly animal tissues, and without definition of a reference culture set-up (1).

Aim of the study is to develop 3D tissue models that simulate osteochondral defects in vitro, to be used to assess the regenerative potential of biomaterials/bank products, cells, growth factors or combination of them.

Cylindrical osteochondral tissue samples were harvested from the femoral head of cadaver beating heart donors. From each sample, a smaller inner portion was extracted; to fully characterize the model, autograft (inner core of the same donor), allograft (inner core from different donor) and empty tissue explants (without inner core) were cultured for 8 weeks in dynamic culture condition.

Osteochondral tissue samples showed stable viability, without significant fluctuations and regardless of the experimental group. Microtomographic images showed signs of integration of the graft, mainly along the lateral contact surfaces between graft and defect. Molecular and histological analyses are in progress to evaluate both regulation of genes related to bone and cartilage metabolism and integration.

The proposed 3D human tissue culture promises to be reliable and replicable, capable of mimicking an osteochondral defect condition for the preliminary evaluation of treatments and therapies.

Keywords

osteochondral defect; in vitro model; osteointegration

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Abstract **#1872**

DEVELOPING NOVEL 3D HYDROGEL-BASED MODELS OF THE HUMAN MAMMARY GLAND TO INVESTIGATE BREAST CANCER INITIATION

Eliana Lingard$_P$[1,2,3], Andrew Gilmore[1], Alberto Saiani[4], Joe Swift[5], Marco Domingos[6], Aline Miller[2,7]

[1] *Division of Cancer Sciences, The University of Manchester*

[2] *Manchester BioGEL*

[3] *UK Regenerative Medicine Platform*

[4] *Department of Materials, The University of Manchester*

[5] *Division of Cell Matrix Biology and Regenerative Medicine, The University of Manchester*

[6] *Department of Mechanical, Aerospace and Civil Engineering, The University of Manchester*

[7] *Department of Chemical Engineering, The University of Manchester*

Corresponding author's email: eliana.lingard@postgrad.manchester.ac.uk

Introduction:

Women with dense breast tissue are at an increased risk of developing breast cancer. This is attributed to the increased periductal collagen stiffness and organisation present in dense breast tissue[1]. The mechanical cues present in stiff breast tissue can stimulate pro-oncogenic behaviour in mammary epithelial cells (MECs) via mechanosensitive signalling pathways[2]. However, these pathways are ill-defined.

Investigating mechanosignalling events between MECs and their environment requires a mechanically tunable and defined model of the human mammary gland. Biomaterials currently used to model the mammary gland suffer from limitations that inhibit their ability to recapitulate the native breast environment, highlighting the need for a biomaterial that meets our requirements[3].

Methods:

PeptiGel® Alpha4 (Manchester BIOGEL), a self-assembling peptide hydrogel that supports MEC viability[4], was used. Non-malignant, human MECs (MCF10a) were encapsulated in Alpha4 for 12 days before being probed for markers of acinar development. Modifications made to gels included diluting them with PBS and mixing in laminin 111.

Results:

Alpha4 supports the development of clusters that deposit a laminin-rich basement membrane but lack other markers of acinar development. Functionalised Alpha4 gels yielded softer gels that increased MCF10a viability and cluster formation. However, these modifications did not stimulate acinar development.

Conclusion:

We show that Alpha4 supports MCF10a cluster formation and basement membrane deposition as well as being amenable to chemical and mechanical tuning. These results suggest that, with further optimisation, Alpha4 can be used to make an accurate model of the human mammary gland which will help us define MEC-matrix interactions.

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Abstract **#1873**

3D SYNTHETIC NICHE EFFECT ON MESENCHYMAL STEM CELLS TRANSCRIPTOME

Carolina Testa$_P$[1,2], Stefania Oliveto[3], Francesca Donnaloja[2], Pietro Pinoli[1], Emanuela Jacchetti[2], Roberto Osellame[4], Giulio Cerullo[4], Stefano Biffo[3], Stefano Ceri[1], Manuela Teresa Raimondi[2]

[1] *DEIB, Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milano, Italy*

[2] *Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy*

[3] *INGM, National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi", Milano, Italy*

[4] *Istituto di Fotonica e Nanotecnologie (IFN)-CNR and Department of Physics, Politecnico di Milano, Milano, Italy*

Corresponding author's email: carolina.testa@polimi.it

Mesenchymal stem cells (MSCs) are the most promising source for cell-based therapies, not only for their potentiality in differentiation and tissue regeneration but also for their immunomodulatory and anti-inflammatory effects and their marked trophic activity [1]. In the panorama of three-dimensional culture systems for stem cells expansion, a particular scaffold called NICHOID was developed, an innovative engineered niche fabricated via two-photon polymerization of a negative hybrid photoresist (SZ2080), conceived to emulate the native microenvironment [2]. The ability of NICHOID to maintain stem cells pluripotency without adding exogenous factors have already been assessed [3][4]. Here we resolved to investigate the effect of 3D NICHOID on the whole transcriptome of MSCs. For this purpose, two versions of NICHOID were fabricated, the 3D and a single-layered one, used as flat control. Rat bone marrow MSCs (rBMSCs) were cultured on both structures, their morphology and viability assessed and RNA extracted after 4 days. Later, differential expression and enrichment analyses were carried out. Results showed that rBMSCs were vital in both conditions but displayed a different morphology, which translated into a total of 603 differentially expressed genes and several interesting deregulated pathways. These evidences proved that NICHOID, thanks exclusively to its tridimensional pattern and to the forces it exerts on cells, is able to maintain, beyond pluripotency, other peculiar features of MSCs.

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Abstract **#1874**

IN-SILICO AND IN-VITRO STUDIES OF DRUG-PROTEIN AND DRUG-DRUG INTERACTIONS FOR THE CANCER TREATMENT

Neema Kumari[1], Vikram Dalal[2], Pravindra Kumar[2], Subha Narayan Rath[1]

[1] *Indian Institute of Technology Hyderabad*

[2] *Indian Institute of Technology Roorkee*

Corresponding author's email: bo15resch11004@iith.ac.in

Increased expression of T-type calcium channel (TTCC) in cancer has paved the way for TTCC blockers to be tested for their anticancer activity. Various studies have shown that blocking TTCCs reduces cancer cell viability. TTCC blockers also provide a possible role as an adjuvant. It is well-known that drug-drug interaction can alter the actions of each other or cause adverse effects. Therefore, using in silico study, binding sites of a TTCC blocker and the paclitaxel (PTX)- a tubulin-binding agent, at the TTCC were studied. The combination index was calculated using in vitro assay to study the interaction between the two drugs.

Molecular docking results showed that both drugs could bind with a good binding affinity with the TTCC at the same sites, thus exhibiting competition for the same sites. However, the TTCC blocker exhibited a higher binding affinity as compared to the PTX.

Further, molecular dynamics analysis confirmed that the TTCC blocker bound at the active site of the TTCC and resulted in the formation of the stable protein-ligand complexes. In vitro study showed the antagonistic interaction between the drugs when given together for the combination treatment. However, the sequential treatment, where the TTCC blocker was removed before adding the PTX, reduced cancer cells' viability further, even at a low TTCC blocker's concentration. Hence, we conclude that the TTCC-TTCC blocker complex is more stable than the TTCC-PTX complex, and sequential treatment can be used to circumvent the competitive antagonistic interaction between the two drugs.

Keywords

Paclitaxel; T-type calcium channel blocker; antagonistic interaction

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Abstract **#1875**

3D-PRINTING OF PLGA/ALGINATE COMPOSITE SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING

Anushree Ghosh Dastidar$_P$[1], Krishna Manda[1], Susan Clarke[1], Fraser Buchanan[1]

[1] *Queens University Belfast*

Corresponding author's email: aghoshdastidar01@qub.ac.uk

Osteoarthritis is a degenerative condition of the Articular Cartilage (AC) occurring due to age, trauma, or repetitive injury. The current treatment provides short-term benefits that have given rise to Tissue Engineering. 3D-printing has surfaced as a promising strategy to engineer mechanically functional scaffolds for cartilage tissue engineering. An advantage of 3D-printing is that the architecture of the scaffold can be controlled and can be mechanically reinforced with networks of stiffer polymers such as Polylactic-co-glycolic acid (PLGA) to engineer composites that are both mechanically functional and supportive of new tissue deposition by resident cells. PLGA has gained considerable interest due to its biocompatibility, tailored biodegradation for cartilage regeneration, and so on. However, synthetic polymers have poor cell adhesion properties which are overcome by natural polymers. Alginate is a naturally derived polysaccharide and a versatile biomaterial owing to its excellent biocompatibility, printability, and ability to support the differentiation of encapsulated cells. Here, a new class of functionalized alginate and PLGA have been developed for the 3D-printing of composite scaffolds. The scaffold loaded with Mesenchymal cells are also found capable of supporting robust chondrogenesis. Immunohistochemical analyses of the scaffolds revealed enhanced type II collagen fibril and cartilage tissue formation. Finally, it is demonstrated that this network of composite 3D-printed PLGA fibres with Alginate showcased a compressive modulus comparable to the native AC. In conclusion, an innovative 3D-printed composite scaffold of PLGA infused with Alginate for cartilage regeneration has been presented.

Keywords

cartilage regeneration; tissue engineering; PLGA

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Abstract **#1876**

3D COLLAGEN-BASED SCAFFOLD MODELS FOR INVESTIGATION OF EPITHELIAL-MESENCHYMAL TRANSITION IN BREAST CANCER

Elizabeth Sainsbury$_P$[1], Fergal J O'Brien[1,2,3], Caroline M Curtin[1,2,3]

[1] *Tissue Engineering Research Group (TERG), Department of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland.*

[2] *Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland*

[3] *Trinity Centre of Bioengineering (TCBE), Trinity College Dublin (TCD), Dublin, Ireland*

Corresponding author's email: elizabethsainsbury@rcsi.com

Treatment options for triple-negative breast cancer (TNBC) are limited to chemotherapy (1). Current cancer models include 2D cell culture and mice models but are unable to accurately mimic human TNBC (2). This highlights the need for the development of a representative in vitro model to study TNBC behaviour and identify new treatment targets. This project aims to develop a collagen-based model composed of components of breast tissue including hyaluronic acid (HyA) and chondroitin sulphate (CS), both elevated in tumours, to investigate their role in the epithelial-mesenchymal transition (EMT) process, which promotes TNBC cell migration and invasion.

Collagen-based slurries comprised of three different concentrations of HyA or CS were freeze-dried and crosslinked using previously optimised protocols (3). Scaffold characterisation was performed using SEM, porosity, pore size analysis and mechanical testing. AlamarBlue, dsDNA and live/dead assays were performed to assess the metabolic activity and growth of TNBC cell lines, MDA-MB-231 and MDA-MB-436. The effect on EMT is being assessed using qPCR and western blot analysis.

Results showed that all scaffolds exhibited characteristics conducive to cell growth. Different concentrations of HyA or CS did not affect cellular growth though cells were more metabolically active in HyA versus CS scaffolds. The effect on EMT is currently being analysed.

In conclusion, we have successfully fabricated suitable scaffolds to model different TNBC tumour microenvironments and assess the EMT process. These scaffolds have huge potential to be used as efficient 3D drug testing platforms.

This work was funded by the Health Research Board Investigator Led Project [ILP-POR-2019-023].

Keywords

3D collagen-based models ; Triple negative breast cancer ; Epithelial-mesenchymal transition

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Abstract **#1877**

SPHEROIDS AS VASCULARIZED BUILDING BLOCK UNITS FOR BONE TISSUE ENGINEERING

Filipa Teixeira$_P$[1], Lorenzo Moroni[1], Carlos Mota[1]

[1] Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration Department, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands

Corresponding author's email: f.castroteixeira@maastrichtuniversity.nl

Spheroids represent an attractive building block unit for bone tissue engineering (TE), especially when pre-vascularized. These allow to create and mimic the in vivo 3D microenvironment, mainly the cell-to-cell and cell-to-extracellular matrix (ECM) interactions and fusion. It is precisely this fusion capacity that makes spheroids a promising building block for bottom-up TE approaches. Bioprinting is one of the approaches where tissue spheroids have been largely investigated to form 3D complex-shaped constructs for tissue and organ regeneration [1]-[4]. The ability to promote adequate vascular morphogenesis in spheroids is a complex process, which is still not completely understood.

In this study, we aim to use vascularized spheroids for bone TE, and evaluate the potential of endothelial cells and their role in osteogenic differentiation. First, we investigated the culture conditions to generate vascularized spheroids using human mesenchymal stromal/stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs). Spheroid fusion in suspension or in hydrogels were investigated to access the assembly into a larger tissue intermediates to form 3D complex vascularized macro-tissue. Results show that the hMSCs/HUVEC ratio strongly influences the stability and compactness of microtissues. Furthermore, cell viability is largely influenced by the ratio and size of the microtissues. Future studies will aim at inducing differentiation to these microtissues and their bioprintability for the creation of vascularized bone implants.

Keywords

Spheroids; Vascularization; Bone tissue engineering

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Abstract **#1878**

ALIGNED MICROGELS CONSTRUCTS FOR 3D PHYSICAL GUIDANCE OF MUSCLE TISSUE DEVELOPMENT

Riccardo Rizzo$_P$[1], Angela Bonato[1], Xhem Qabrati[2], Ori Bar-Nur[2], Marcy Zenobi-Wong[1]

[1] Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences & Technology, ETH Zürich, Otto-Stern-Weg 7, 8093 Zürich, Switzerland

[2] Laboratory of Regenerative and Movement Biology, Department of Health Sciences and Technology, ETH Zürich, Schorenstrasse 16, 8603 Schwerzenbach, Switzerland

Corresponding author's email: riccardo.rizzo@hest.ethz.ch

In recent years, the design of photoactivatable materials has stimulated a widespread use of light-mediated biofabrication techniques,1 which present powerful tools to mimic multiscale tissue organization, with resolution ranging from nanometer to centimeter. However, most of the work has been done with homogenous, bulk hydrogel compositions, hence overlooking the potential of three-dimensional microarchitecture in directing cell fate. In certain tissues, such as muscle, cell alignment plays a fundamental role in physiological tissue function. Our group has developed a simple method to create microarchitected hydrogels,2 which provide physical guidance for oriented cell growth and tissue development and a vastly improved mass transport, therefore helping to overcome nutrient diffusion, a major and common obstacle for the realization of centimetre-sized grafts. Aligned microgels are generated by sizing a cell-laden bulk hydrogel through grids with apertures ranging from 20 to 100 µm. A second light-driven crosslinking step stabilize the aligned microstructures. Here we first report C2C12 myoblasts fusion and maturation in aligned myofibers, resulting in a contractile construct. In addition, we investigated the influence of various size of Gel-MA based microgels (20, 40, 70 and 100 µm) on the oriented and functional maturation of wild type primary mouse myoblasts. The importance of cellular crosstalk in the mature and contractile construct was also assessed by the introduction of other cell types, such as endothelial and satellite cells. Finally, the potential use of this system as an in vitro disease model has been evaluated with myoblasts from a mouse model of Duchenne Muscular Dystrophy.

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Abstract **#1879**

EVALUATION OF THE ODONTOGENIC POTENTIAL OF HUMAN PERIODONTAL LIGAMENT CELLS SUBPOPULATIONS IN VITRO

Esteban Astudillo-Ortiz[P][1,2,3], Pedro S Babo[1,2], Ana I Gonçalves[1,2], Rui L Reis[1,2], Manuela E Gomes[1,2]

[1] 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal

[2] ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães 4805-017, Portugal

[3] Department of Endodontics, Area of Histology, School of Dentistry, University of Cuenca, Ecuador

Corresponding author's email: esteban.astudillo@ucuenca.edu.ec

Dental pulp (DP) and periodontal ligament (PDL) cells derive embryologically from the interaction between cells from the neural crest and the underlying mesenchyme. Given the limited availability of dental pulp cells (DPCs), there is a need to find alternative cells sources for endodontic tissue engineering applications. Mesenchymal stem cells (MSCs)-like populations have been isolated from both DP and PDL. However, these population are very heterogenous and present dissimilar differentiation potential.

This study aimed at evaluating the odontogenic potential of 3 specific subpopulations (SSEA-4+, CD90+, and nestin+), isolated from human PDL fibroblasts (PDLFs) using magnetic sorting. These cells were cultured on basal and osteogenic α-MEM for 7 and 14 days. Cultures were assessed in terms of proliferation, ALP activity, mineral matrix deposits, calcium content, and gene expression.

Results showed that the SSEA-4+ cell population was more prone to differentiate into the odontoblast phenotype and deposit more mineralized matrix compared to a non-sorted population or DPCs from the same donor.

We concluded that the SSEA-4+ PDLF cells population could differentiate into the odontogenic pathway, whereas CD90+ and nestin+ PDLFs were more similar to the original fraction. Furthermore, our studies show that SSEA4+ cells can be homed directly from bulk PDLFs into hydrogels using magnetized files. Overall, these results allow us to propose SSEA-4+ PDLFs as a potential alternative to DPCs for tissue engineering approaches aiming endodontic treatments.

Acknowledgements:

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Keywords

Endodontic tissue engineering; Periodontal cells subpopulations; Odontoblast differentiation

Abstract **#1880**

A NOVEL BIOENGINEERED TISSUE MODEL FOR THE DEVELOPMENT OF PHARMACEUTICALS IN THE TREATMENT OF HUMAN INFLAMMATORY BOWEL DISEASE

Claire L Mobbs[P][1,2], Stefan A Przyborski[1,2]

[1] *Durham University*

[2] *Reprocell Europe*

Corresponding author's email: claire.l.mobbs@durham.ac.uk

Inflammatory bowel diseases (IBD) are complex pathologies with an increasing worldwide prevalence. It is therefore essential to better understand the cellular interactions underpinning these diseases. However, modelling IBD in vitro is difficult due to their complex aetiology. The scarcity and short-lived nature of IBD tissue ex vivo, low similarity between animal and human tissue, and absence of physiologically relevant in vitro models impedes research progression.[1][2] Most research efforts have focused on immune-mediated barrier damage, neglecting other critical mediators of the inflammatory response, including fibroblasts. There exists irrefutable evidence that fibroblasts are not just bystanders in mucosal inflammation but are pivotal participants in orchestrating the immune response.[3]

We have bioengineered a highly reproducible, in vitro model that better recapitulates aspects of the IBD intestinal mucosa. These constructs utilise a porous polystyrene scaffold to construct lamina-propria-like compartments with an overlying epithelium. Subepithelial fibroblasts within the lamina-propria secrete endogenous ECM, alongside a co-cultured viable immune component that simulates the mass immune-cell infiltration observed in IBD.

These IBD models can be treated with stimuli to produce an inflammatory environment. Mimicking an inflammatory phenotype induces endogenous cytokine secretion, impacts barrier function, as well as stimulating lamina-propria inflammatory events such as ECM-remodelling. This in vitro system allows for investigation into complex interplay between epithelial, mesenchymal and immune cells and the resultant effects on barrier function, thus simulating key aspects of IBD. These results demonstrate the potential for this novel bioengineered system to be used for pharmaceutical compound testing in a controlled, reproducible and physiologically relevant environment.

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Abstract **#1881**

ANISOTROPY AND INHOMOGENEITY IN HUMAN LATERAL MENISCI: MECHANICAL AND HISTOLOGICAL EVIDENCE

Gregorio Marchiori[1], Matteo Berni[1], Melania Maglio[1], Giorgio Cassiolas[2], Alberto Grassi[1], Andrea Visani[1], Stefano Zaffagnini[1], Milena Fini[1], Nicola Francesco Lopomo[2]

[1] *IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy*

[2] *Università degli Studi di Brescia, Brescia, Italy*

Corresponding author's email: gregorio.marchiori@ior.it

Human meniscus presents a peculiar time-dependent response to loads - which is related to the interaction between solid (extra-cellular matrix with collagen fibres network) and fluid phase -, that makes it a key player in knee biomechanics. Although the importance of meniscus pathophysiology, a complete assessment of the relation between structure and function is not yet achieved. Thus, the purpose of this study was to elucidate the contribute of solid and fluid phase in response to compressive load. Mechanical tests were performed on cubic samples, harvested from anterior horn, central body and posterior horn of 7 human lateral menisci, where radial, circumferential and vertical directions were clearly identified. Fibril-network-reinforced biphasic model was fitted to experimental data achieved through unconfined compression tests, in order to estimate fibril modulus Ef, matrix modulus Em and hydraulic permeability k of the tissue. Histological analyses were performed taking care to visually maintain the information about the orientation adopted during mechanical test. Tissue porosity and collagen fibres arrangement were evaluated. Regional and strain-dependent parameters for a fibril-reinforced biphasic model of the lateral meniscus tissue were proposed, specifically isotropic in the horns and transversely isotropic (i.e. isotropy plane formed by radial and vertical directions) in the body. Fibres orientation and percentage of porosity on various regions and planes supported the differences highlighted in the compressive responses. The mechanical and histological observations of this study support the functional characterization of the meniscal tissue, crucial for modelling the tibio-femoral contact and for the realization of constructs for meniscal replacement.

Keywords

Meniscus; Compression; Histology

Abstract **#1882**

EVALUATION OF TENDON-DERIVED EXTRACELLULAR MATRIX (TDECM) IN A MICROFLUIDIC DEVICE: TOWARDS TISSUE-SPECIFIC 3D MATRIX ON CHIP.

Akshay Bhatt$_P$[1], Nandini Dhiman[1], Falguni Pati[2], Subha Rath[1]

[1] *Regenerative Medicine and Stem Cell Laboratory, Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, NH-65, Kandi, Sangareddy 502285, Telangana, India.*

[2] *Biofab TE laboratory, Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, NH-65, Kandi, Sangareddy 502285, Telangana, India.*

Corresponding author's email: akshaybhatt@iith.ac.in

The decellularized extracellular matrix (dECM) is used as a bioink for 3D bioprinting or as a hydrogel for studying the physiology of tissues. In this study, we obtained dECM from goat digital flexor tendon using physical and chemical methods. The dECM was subjected to enzymatic digestion and the final hydrogel was termed as tendon-derived ECM (tdECM). The tdECM was characterized for its physicochemical properties using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Collagen, dsDNA, GAGs, and protein contents were quantified using spectrophotometric assays. The cell viability and proliferation of mouse fibroblast cells (L929) and human umbilical cord-derived mesenchymal stem cells (UMSCs) encapsulated in the tdECM hydrogel inside the microfluidic device was checked using Calcein-AM/PI. The FTIR data showed prominent peaks of amide group indicating the presence of collagen. The SEM data showed intact fiber morphology after the decellularization process. There was 94% reduction in double-stranded DNA (dsDNA) content, hence, proving the effectiveness of the decellularization technique. There was no significant difference in the collagen content of tdECM as compared to the native tissue. The GAGs and protein contents were reduced due to the decellularization process but were in the acceptable range. Over 90% cell viability in L929 and UMSCs was observed both qualitatively and quantitatively. In conclusion, we demonstrate the effectiveness of the decellularization process using a combination of physical and chemical methods followed by tdECM biocompatibility in a microfluidic device to understand the stem cell behavior in a 3D condition.

Keywords

Tendon-derived extra cellular matrix (tdECM); microfluidic model; umbilical cord-derived mesenchymal stem cells (UMSCs)

Abstract **#1883**

CASTING OF HUMAN SIZED MULTI-LAYERED EARS FOR THE TREATMENT OF MICROTIA

Enrico Tosoratti[P][1]

[1] *Tissue Engineering + Biofabrication Laboratory, ETH Zürich, Switzerland*

Corresponding author's email: enrico.tosoratti@hest.ethz.ch

Tissue-equivalent implants are necessary for the treatment of craniofacial deformations such as microtia. However, biofabrication of multi-material constructs remains challenging due to the complex and layered structure of anatomical tissues[1,2].

Additive manufacturing technologies can help in the biofabrication of tissue-equivalent implants. Our technique for the biofabrication of heterogeneous multi-layered implants uses diffusion induced gelation and involves the computational design of multi-part molds, termed metamolds[3], that respect the geometric and topological constraints involved in the process of casting. Metamolds are fabricated using biopolymers and are preloaded with a crosslinker of interest. The subsequent casting of the implant into consecutive metamolds allows for the precise generation of multi-layered hydrogels with elevated precision (50 µm). The mechanical stability and bonding between adjacent layers are achieved using an enzymatic-based strategy triggered by the elution of 100 mM CaCl2 and 0.1% H2O2 from the metamolds. The porosity of the construct is increased by fabricating the core material using entangled microstrands[4] and including sacrificial microstrands in the outermost layers.

As a proof of concept, three-layered anatomical human-sized ears were generated using different biomimetic hydrogels. The cartilage structure was formed using auricular chondrocytes embedded in an HA-tyramine/alginate-based hydrogel. The perichondrium layer was formed using chondroprogenitor cells within a densely crosslinked collagen-tyramine gel. Finally, fibroblasts and endothelial cells were encapsulated within a loose collagen gel to resemble a layer of connective tissue. Samples were cultured for nine weeks and analyzed via mechanical testing and immunochemistry showing excellent cell viability and maturation.

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Abstract **#1884**

INTRA-ARTICULAR TRIAMCINOLONE ACETONIDE-LOADED LIPOSOMES DO NOT AFFECT THE PROGRESSION OF JOINT DAMAGE IN A RAT METABOLICALLY ACCELERATED GROOVE MODEL OF OSTEOARTHRITIS

J. L. Rios[1], H. M. de Visser[1], G. Geusebroek[2], G. Storm[2], N. Dupuis[2], S. G. Plomp[2], K. Warmink[1], N. Eijkelkamp[1], M. Tryfonidou[2], P. R. van Weeren[2], H. Weinans[1], N. M. Korthagen[2,3]

[1] *University Medical Center Utrecht*

[2] *Utrecht University*

[3] *Leiden University Medical Center*

Corresponding author's email: n.m.korthagen@lumc.nl

We evaluated the effect of slow-release anti-inflammatory treatment (triamcinolone acetonide loaded liposomes [TAA-L]) in a model mimicking metabolically accelerated post-traumatic osteoarthritis. Wistar rats were fed a high-fat diet, twelve weeks after diet-onset, bilateral osteoarthritis was induced employing small grooves at the femoral condyles1. At 13 and 19 weeks, rats received an intra-articular injection with TAA-L in their left knee (30µg or 100µg TAA). Saline or empty liposomes were injected intra-articular in the right knee. At week 24, rats were euthanized, and knees were harvested for histology (OARSI score) and microCT images (subchondral plate thickness and osteophytes). Preliminary results show no difference between groups for the OARSI cartilage (average ±SD: 4.7 ±2.3 out of 15; p=0.5) and subchondral bone scores (average ±SD: 1.2 ±0.4 out of 5; p=0.5), nor for subchondral plate thickness (average ±SD: 272 ± 30 µm, p=0.2) in the tibial lateral plateau. Interestingly, none of the rats that received TAA-L injection at a dose of 30 µg presented signs of synovial inflammation (OARSI score 0), while control knees treated with saline, empty liposomes, or 100 µg TAA-L presented signs of synovial inflammation in 50% of the rats. In conclusion, TAA-L did not prevent joint damage progression in our metabolically accelerated groove model of osteoarthritis, but 30µg TAA-L reduced synovial inflammation. Thus, low doses of TAA-L might be a strategy to reduce joint (synovial) inflammation and possibly related joint pain, therefore, further investigation of the potential dose-response analgesic and anti-inflammatory effects is recommended.

Keywords

Osteoarthritis; Liposome; Triamcinolone acetonide

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Abstract **#1885**

ADAPTING NEW OTOLOGIC DEVICES TO THE CLINICAL ENVIRONMENT – THE NEXT GENERATION OF TYMPANIC MEMBRANE REPAIR

Elaine Horn-Ranney[P][1], Sithara Nair[1], Bethany Young[1]

[1] *Tympanogen, Inc.*

Corresponding author's email: elaine@tympanogen.com

INTRODUCTION: Perf-Fix light-curable gel patch is a highly regenerative and non-ototoxic biomaterial intended to replace traditional tympanoplasty with a cost-effective in-office procedure while regenerating native tympanic membrane (TM) architecture, thereby eliminating a common surgery and its complications for thousands of patients annually. Mechanistic, usability, and regulatory design criteria for Perf-Fix were optimized for current clinical workflows for facile transition from bench to patient. METHODS: Custom pouches that fit luer-lock syringes were filled with lyophilized Perf-Fix reagents for intuitive reconstitution with saline. Uncured Perf-Fix was applied to animal and surrogate TMs with a curette, then cured with blue light from a custom curing light. Perf-Fix delivery system designs were refined through user feedback and anatomical simulation. Safety and efficacy were evaluated through ISO 10993 biocompatibility studies. Shelf-life and sterility were evaluated with rheometry, aging and bioburden studies. RESULTS: Cured Perf-Fix formed a semi-interpenetrating network of hyaluronic acid and modified chitosan, and demonstrated no cytotoxicity after curing, amenable storage (2.5-5.0 kPa) and loss (0.2-0.4 kPa) moduli, and superior curing properties with human anatomical simulation testing. User-driven modifications of Perf-Fix, curing light, and delivery system resulted in positive clinician feedback and 90% reduction in procedure time. A sterility assurance level of 10-6 and shelf-life of 1 year by accelerated aging was achieved. CONCLUSIONS: Clinical translation requires constant user input with clear regulatory and implementation strategies from ideation. The adaptation of Perf-Fix to seamlessly fit clinical workflow may significantly improve tympanoplasty by eliminating the surgical aspect of TM repair and improve treatment outcomes.

Abstract **#1886**

BIOPRINTING OF MICROTISSUES FOR OSTEOCHONDRAL TISSUE ENGINEERING

Ane Albillos Sanchez[P][1], Lorenzo Moroni[1], Carlos Mota[1]

[1] Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration Department, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands

Corresponding author's email: a.albillossanchez@maastrichtuniversity.nl

Osteoarthritis (OA) is a degenerative joint disease that affects 1 in 3 people over 65 years old (1). OA established treatments often lead to complications and fail to deliver long-lasting satisfactory results. Therefore, tissue-engineering approaches aim to obtain de novo tissues to aid in the treatment of this disease (2). 3D bioprinting holds great promise in the generation of osteochondral constructs because it allows to deposit living cells combined with biomaterials in a layer-by-layer fashion (3). When combined with spheroids in a bottom-up approach, this technology has the potential to produce joint implants with the inbuilt biological information to produce osteochondral tissue in a process that mimics the endochondral ossification route.

In our work, we investigated hyaluronic acid as a biomaterial ink to form a spheroid-based bioink for bioprinting of osteochondral constructs. For this, hyaluronic acid was successfully methacrylated (HAMA), combined with Lithium phenyl-2,4,6-trimethyl-benzoylphosphinate (LAP) and photocrosslinked. Human mesenchymal stem/stromal cell spheroids (hMSC) were encapsulated in the synthesized biomaterial. Cell viability and distribution were assessed after one and seven days in culture, showing good cell viability and spheroid-to-spheroid fusion capacity within the biomaterial. Biomaterial inks were used to produce bioprinted scaffolds, and structural stability and resolution were optimized investigating the processing parameters. Ongoing and future experiments include osteochondrogenic differentiation analysis of encapsulated and bioprinted constructs. Furthermore the optimization of bioink formulations will take into account bioprintability, spheroid fusion allowance and degradation capacity. Finally, human periosteum derived cells will be studied as an alternative cell source for osteochondral implant manufacturing.

Keywords

Bioprinting; Microtissues; Joint

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Abstract **#1887**

THE USE OF CERAMIC-MODIFIED POLYESTER BLENDS FOR THE MANUFACTURE OF A MULTI-FUNCTIONAL BILAYER MEMBRANE FOR THE REGENERATION OF PERIODONTAL TISSUE

Ali Alqahtani[P][1], Andrew A. Gill[2], Robert Moorehead[3], Ilida Ortega Asencio[4]

[1] Bioengineering and Health Technologies Group, The School of Clinical Dentistry, University of Sheffield, Sheffield S10 2TA, UK.

[2] Floreon- Transforming Packaging Ltd, G9, Ergo, Bridgehead Business Park, Hessle, HU13 0GD

[3] The Henry Royce Institute and Department of Materials Science and Engineering, The University of Sheffield, Sir Robert Hadfield Building, Sheffield, S1 3JD, United Kingdom

[4] Bioengineering and Health Technologies Group, The School of Clinical Dentistry, University of Sheffield, Sheffield S10 2TA, UK

Corresponding author's email: amalqahtani1@sheffield.ac.uk

Background: Guided Bone Regeneration (GBR) is a regenerative therapeutic procedure that has been widely studied for its effective role in treating periodontal disease. Different membranes are currently used for GBR, however, none of them are completely fit for purpose(1). Researchers at Sheffield have partnered with Floreon Ltd. to test the biomedical potential of using a new polyester blend to manufacture an innovative GBR membrane.

Objective: The aim of this study was to fabricate a bilayer membrane made of ceramic-modified Floreon and to evaluate its mechanical and physicochemical performance.

Methods: A bi-layered membrane was fabricated which consisted of(i) porous bottom layer using electrospinning and Floreon reinforced with 10% of ceramic components; we hypothesise this membrane would preserve the bone socket; (ii) smooth layer of pure Floreon on top (physical barrier) obtained by spin-coating technique. PLA membranes were also produced as controls. Characterisation was performed for biophysical and mechanical properties through SEM, AFM, (DSC), porosity, wettability, tensile testing. The degradation was also monitored.

Results: The bilayer nanofibrous membranes exhibited excellent mechanical properties initially as well as after one month in PBS; Moreover, the electrospun Floreon membranes reinforced with ceramics exhibited an increase in pore diameter and fibre size which satisfies the ideal properties needed for GBR membranes.

Conclusion: Our combination of manufacturing techniques has proven to be a promising method for the development of new GBR membranes. This work demonstrates the potential of using Floreon and ceramic-modified Floreon for dental applications, and the advantages of modifying these blends with a Bio-ceramic components.

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Abstract **#1888**

REINFORCED COLLAGEN BASED SCAFFOLDS FOR MUSCULOSKELETAL TISSUE ENGINEERING

Mark Lemoine$_P$[1,2], John O'Byrne[1,3], Daniel Kelly[1,2,4], Fergal O'Brien[1,2,4]

[1] *Tissue Engineering Research Group, Dept. of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin*

[2] *Trinity Centre for Bioengineering, Trinity College Dublin (TCD), Dublin*

[3] *Department of Trauma and Orthopaedic Surgery, Royal College of Surgeons in Ireland (RCSI), Dublin*

[4] *Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin*

Corresponding author's email: marklemoine@rcsi.ie

INTRODUCTION: Regeneration of osteochondral tissue presents a clinical challenge as its layered structure has depth varying mechanical properties and composition. Our lab has developed collagen scaffolds that can regenerate osteochondral tissue but their mechanical properties restrict their use to small joint defects [1,2]. To overcome this limitation, the aim of this study was to develop a biomimetic scaffold with mechanical properties suitable to restore function in large joint defects. METHODS: 3D-printed polycaprolactone scaffolds mimicking the mechanical properties of the of native tissue was combined with a tri-layered collagen type I based matrix with a chondral layer with an addition of collagen type II and hyaluronic acid (HyA), an intermediate layer with addition of HyA, and a osteo layer with an addition of nano-hydroxyapatite. RESULTS: The scaffolds' compressive modulus increased from 0.5MPa in the chondral layer to 16.5MPa in the osteo layer, and had high fixation (47.4kPa) and shear (0.93MPa) strengths to ensure that it can withstand the forces in an articulating joint. The scaffolds supported layer-specific stem cell differentiation, with cartilage matrix formation in the chondral layer and mineralization in the osteo layer. Mechanical properties of the scaffolds increased with culture time, proving that functional osteochondral restoration is possible with this scaffold. SIGNIFICANCE: This novel tri-layered scaffold was shown to support layer-specific stem cell differentiation in vitro, with mechanical properties suitable for load bearing joints to regenerate large defects to alleviate the need for joint replacement surgeries. In vivo assessment in an osteochondral defect model in goats is currently ongoing.

Keywords

Collagen based scaffolds; Osteochondral regeneration

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Abstract **#1889**

DEVELOPMENT OF A NOVEL SMART WOUND DRESSING: A STRATIFIED PNIPAAM HYDROGEL-EGGSHELL MEMBRANE HYBRID INCORPORATING DRUG LOADED NANOPARTICLES

Emily Briggs[P][1], Nik Aliaa[2], Lady Barrios Silva[3], Rosemond Atta Mensah[3], Linh Nguyen[3], David Chau[3]

[1] *Division of Medical Sciences, University College London, London, United Kingdom*

[2] *Division of Biomaterials and Tissue Engineering UCL Eastman Dental Institute University College London London, United Kingdom*

[3] *Division of Biomaterials and Tissue Engineering UCL Eastman Dental Institute University College London London, United Kingdom*

Corresponding author's email: emily.briggs.17@ucl.ac.uk

Damage or degeneration of any (biological) membrane is often as a consequence of disease and/or traumatic injury and current therapeutic treatments suffer from a number of limitations. The chicken eggshell membrane (ESM) is a unique material: not only does its innate physical and mechanical profile confer optimal barrier properties, it also demonstrates inherent biocompatibility/biodegradability characteristics. Moreover, to further enhance its therapeutic potential, the ESM may be further modified with the thermo-responsive polymer, poly(N-isopropylacrylamide) (PNIPAAm) as well as the incorporation of (drug-loaded) nanoparticles (NP): essentially, by changing the temperature, the release and delivery of NP can be targeted and controlled.

ESM samples were isolated using an optimised acid decellularization protocol and the physical and mechanical characteristics assessed using DMA, SEM, WCA, FT-IR and TGA/DSC. In vitro biocompatibility and cytotoxicity were performed using fibroblast cell lines (i.e. Malme-3, Malme-3M and 3T3) and standard cell culture assays. Optimisation of PNIPAAm hydrogel and NP formulations were identified before being combined with the ESM. Thereafter, additional mechanical and physical characterisation including drug loading, release and diffusion were performed on this construct.

ESM samples were successfully prepared and fully characterized. Fibroblasts cultured on both the extracted ESM samples and ESM-gel demonstrated high biocompatibility in terms of high cell attachment, spreading, viability and proliferation rates. NP were successfully loaded into construct and demonstrated a desirable release profile depending on the specific formulation (days to weeks). As such, this work summarizes the development of an ESM-based construct that may have significant impact in regenerative medical applications.

Keywords

Eggshell membrane; Thermoresponsive; Skin

Abstract **#1890**

DEVELOPMENT OF 3D CARDIAC MODELS VIA MAGNETIC MANIPULATION FOR DRUG SCREENING STUDIES

Rabia Onbasp[1], Ahu Arslan Yildiz[1]

[1] *Department of Bioengineering, Izmir Institute of Technology (IZTECH), 35430 Izmir, Turkey*

Corresponding author's email: rabiaonbas@gmail.com

Drug discovery and development process comprise of preclinical and clinical phases that are very intensive, long, and expensive research phases. However, drug candidates can fail in clinical trials. Toxicity is the major reason that leads to about 30% of drug development failures. Recently, the withdrawal rate of drugs from the market was increased to 33.3% from 5.1% due to cardiotoxicity. When the drug fails at phase I, the reasons are probably related to 2-dimensional (2D) cell culture studies that do not represent the real tissue physiology; therefore, they provide misdirected data about the efficacy and toxicity of drug. On the other hand, cells in 3D cell culture give responses more alike cells in vivo conditions in terms of cell morphology, growth, proliferation, migration, and drug sensitivity [1]. For that reason, 3D cell culture is a promising approach to overcome problems of conventional methods at the preclinical phase. To create 3D cell culture models, cell manipulation techniques have been used for various tissue-engineering applications. Contactless magnetic manipulation techniques provide rapid, simple, and cost-effective 3D cell culture model formation where either paramagnetic agent [2-5] or magnetic materials [6] were utilized. We have developed 3D cardiac model based on a contactless magnetic manipulation approach to investigate doxorubicin-induced cardiotoxicity. This technique provides an easy and efficient way to fabricate 3D cardiac cellular structures for drug screening studies compared to conventional methodologies.

Keywords

contactless magnetic manipulation; cardiac tissue engineering; drug screening

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Abstract **#1891**

GENOME WIDE TRANSCRIPTOME PROFILING ANALYSIS OF TRABECULAR MESHWORK PROGENITOR CELLS: THE FIRST STEP TO A CELL-BASED THERAPY TO RESTORE THE TRABECULAR MESHWORK IN GLAUCOMA

Xiaochen Fan[P][1,2,3,4], Emine Bilir[1,2,3,4], Stephnie Kennedy[1,2,3,4], Victoria Kearns[1,2,3,4], Colin Willoughby[5,6,7,4], Carl Sheridan[1,2,3,4]

[1] *Department of Eye and Vision Science*

[2] *University of Liverpool*

[3] *Liverpool*

[4] *United Kingdom*

[5] *School of Biomedical Sciences*

[6] *Ulster University*

[7] *Northern Ireland*

Corresponding author's email: Xiaochen.fan@liverpool.ac.uk

Introduction

Loss and dysfunction of trabecular meshwork (TM) cells occurs with increasing age and is accelerated in primary open-angle glaucoma (POAG). TM progenitor cells (TMPCs) have the potential to repopulate the TM and could be used as a cellular therapy to restore the glaucomatous TM function. The biological properties and specific markers of TMPCs are still elusive. Understanding the transcriptome of TMPCs will identify specific markers and biological properties to facilitate the development of cell-based therapies to restore TM function in POAG.

Methods

Genome-wide transcriptome profiling was performed using RNA-seq of human primary TM cells (PTM), TMPCs and their differentiated cells (DTM) and analysed by using bioinformatics analyses. The differentially expressed genes (DEGs) of the three cell types were confirmed by NanoString.

Results

TMPCs proliferated, formed spheres, and could differentiate to TM cells in vitro. NanoString confirmed the RNA-Seq data and PLTP, PROS1, TIMP1 and MMP14 mRNA expression were increased in TMPCs compared with PTM cells. The IPA results identified that KDR, IGF1, FOS and MMP9 genes were nodal genes in the development of TMPCs. The activated pathways in the TMPCs which were related to the neuronal cell development.

Conclusion

TMPCs can be harvested and differentiated into the TM cells from human explant cultures in vitro. PLTP, PROS1, TIMP1 and MMP14 genes represent cellular markers for TMPCs. The pathways activated in TMPCs were consistent with the development of neuronal and endothelial cells. Understanding the key genes and pathways controlling TMPC biology are key to developing cell-based therapies for glaucoma.

Keywords

Glaucoma; Trabecular meshwork; Stem cell therapy

Abstract **#1892**

BONE-TARGETING DELIVERY OF ALENDRONATE FOR THE TREATMENT OF OSTEOPOROSIS

Chaozong Liu[P][1], Shenmao Chen[1], Lingling Zhao[1,2]

[1] *University College London*

[2] *Ningbo University*

Corresponding author's email: chaozong.liu@ucl.ac.uk

BACKGROUND & AIMS:

Osteoporosis is a major health burden. Current therapeutic treatments have disadvantages such as systemic side effect and low bioavailability. Bone-targeting drug delivery system are designed to improve the therapeutic effect of drugs and minimize the potential toxic side effects. We fabricated a novel drug nanocarrier for bone-targeting alendronate delivery using glycol chitosan (GC)-poly(lactide-co-glycolide) (PLGA) and PLGA-alendronate conjugates.

MATERIALS & METHODS:

Chitosan-based nanoparticles were prepared with GC-PLGA and PLGA-alendronate conjugate by nanoprecipitation. Alendronate sodium, a commonly used bisphosphonate drug for osteoporosis therapy, serves as both a bone-targeting ligand and a loading drug. The size of the nanoparticles was determined by dynamic light scattering (DLS) measurement. The morphology of the GC-PLGA/PLGA-alendronate nanoparticles was examined by scanning electron microscopy (SEM). Drug release profile, cytotoxicity and cellular uptake were evaluated in vitro. Bone-targeting potential was assessed by hydroxyapatite binding assay and an ex vivo porcine bone model.

RESULTS:

The conjugation of GC-PLGA and PLGA-alendronate was confirmed by Fourier-transform Infrared Spectroscopy (FTIR) and nuclear magnetic resonance (NMR) analysis. The prepared nanoparticles are highly aqueous dispersible with an average size range from 90 to 130 nm based on various polymer ratio. Morphological characterization (SEM) revealed that the nanoparticles are spherical in shape. In vitro tests demonstrated sustained drug release of alendronate, good biocompatibility and intracellular uptake of nanoparticles. Hydroxyapatite affinity test and ex vivo porcine bone model confirmed the bone-targeting potential.

CONCLUSION:

The bone-targeting GC-PLGA nanoparticles may be a drug delivery system for the treatment of osteoporosis. Further study of biodistribution is warranted.

Abstract **#1893**

TOWARDS THE DEVELOPMENT OF A BIOARTIFICIAL KIDNEY USING POLYHYDROXYALKANOATES

Syed Mohammad Daniel Syed Mohamed$_P$[1], Gavin Welsh[2], Ipsita Roy[1]

[1] *Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK*

[2] *Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK*

Corresponding author's email: smdsyedmohamed1@sheffield.ac.uk

Acute kidney injury (AKI) and chronic kidney disease (CKD) are two common kidney problems where the kidney loses its blood filtration ability resulting from the deterioration of glomerulus.[1] Current approaches used to address these conditions include haemodialysis, stem cell therapy[2] and kidney transplantation. However new approaches and therapies are urgently required to diminish morbidity and mortality in patients with these diseases. Recent advances in tissue engineering technologies have led to the opportunity to develop a bioartificial kidney utilising human kidney cells to restore both filtration functionality and innate physiological activities.

This work involves the development of a potential bioartificial kidney using 3D printing (Fused Deposition Modelling). Polyhydroxyalkanoates (PHA), a family of bacteria-derived polymers, have been chosen as the main structural biomaterial. PHAs are highly biocompatible, have versatile mechanical properties, and are bioresorbable in the human body.[3] There are two types of PHAs, short-chain length PHAs, scl-PHA (monomer chain length C4-C5) and medium-chain length PHAs, mcl-PHAs (monomer chain length C6-C16). A specific type of elastomeric PHA, Poly(3-hydroxyoctanoate-co-3-hydroxydecanoate) has been chosen due to its mechanical properties being similar to that of kidney tissue, biocompatibility, and printability. Human conditionally immortalised podocytes (CIHP) have been initially used as the cell type.[4] A biomimetic construct has been developed as a first step forward towards the development of a mature bioartificial kidney. This construct is designed to mimic the glomerulus in the kidney. Ultimately, we aim to create a wearable kidney that will be able to replace an impaired kidney, maintaining the original physiological potency.

Keywords

Bioartificial Kidney; Polyhydroxyalkanoates; 3D Printing

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Abstract **#1894**

OSTEOCHONDRAL SCAFFOLD INNOVATION FOR EARLY INTERVENTION OF CARTILAGE DEFECT: FROM BENCH TO CLINIC

Chaozong Liu[P][1]

[1] *University College London*

Corresponding author's email: chaozong.liu@ucl.ac.uk

OA is a whole joint disease and affects both cartilage and the underlying subchondral bone. However, the spatial relationships between cartilage lesion severity and microstructural changes in the subchondral plate and trabecular bone remain elusive.

Femoral heads from hip arthroplasty for primary osteoarthritis and femoral neck fracture cases were used in this study. Scans of each femoral head were divided into 4 quadrants followed by morphometric analysis of subchondral plate and trabecular bone in each quadrant. Principal component analysis (PCA) was employed to assess differences between OA and non-OA samples, and the spatial relationship between CLS and subchondral bone changes.

Mapping of the trabecular bone microstructure in OA patients with low CLS revealed trabecular organisation resembling non-OA patients, whereas clear differences were identifiable in subchondral plate architecture. The OA-related changes in subchondral plate architecture were summarised in the first principle component (PC1) which correlated with cartilage lesion severity in all quadrants, whilst by comparison such associations in trabecular bone were most prominent in the higher weight-bearing regions of the femoral head. Greater articular cartilage deterioration in OA was regionally-linked with lower BV/TV, TMD and thickness, and greater BS/BV and porosity in the subchondral plate; and with thinner, less separated trabeculae with greater BMD and BS/BV in the trabecular bone.

Our findings suggest that impairment of subchondral bone microstructure in early OA is more readily discernible in the cortical plate and that morphological characterisation of the femoral head bone microstructure may allow for earlier OA diagnosis and monitoring of progression.

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Abstract **#1895**

IONICALLY ANNEALED ZWITTERIONIC MICROGELS FOR BIOPRINTING OF POROUS STRUCTURES

Frantisek Surman[P][1], Maryam Asadikorayem[1], Patrick Weber[1], Marcy Zenobi-Wong[1]

[1] Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zürich, CH-8093 Zürich, Switzerland

Corresponding author's email: frantisek.surman@hest.ethz.ch

3D bioprinting of clinically-relevant tissues requires a sufficient supply of nutrients to ensure cell survival, which is still a significant challenge.[1] Conventional hydrogels used in tissue engineering have two major limitations. Firstly, precursor solutions form a nanoporous hydrogel upon gelation, which greatly limits diffusion of nutrients, leading to reduced cell viability in the center of large grafts. Secondly, upon in vivo implantation, biomaterials are often encapsulated within a fibrous collagenous capsule.[2,3] To address both these limitations, microgel materials made of zwitterionic hydrogels were used to both provide a more permissive macroporous environment for cells and to evade foreign body reactions.[4,5] Here, we present a novel approach to bioprint zwitterionic microgels designed from the low molecular weight carboxybetaine acrylamide (CBAA). CBAA was photopolymerized using alginate methacrylate as a bifunctional crosslinker to produce a bulk hydrogel. Microgels were then mechanically fragmented using progressively fine steel meshes (500 µm to 35 µm pore sizes). Rheology revealed that swollen poly(CBAA) microgel bioinks showed both shear-thinning and shear-recovery properties optimal for extrusion bioprinting. Bioprinted structures were rapidly annealed in the presence of calcium at mild and cytocompatible conditions and showed excellent mechanical stability in cell culture medium. Human chondrocytes encapsulated in the granular material showed highly viable (>90%) during in vivo culture, suggesting that hydrogels were biocompatible. These macroporous and non-immunogenic bioinks show great promise for in vivo applications of bioprinted tissues and organs.

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Abstract **#1896**

ALLOGENEIC TENOCYTE DERIVED EXOSOMES IMPROVE WOUND CLOSURE IN VITRO

Devin von Stade$_P$[1], Kirk McGilvray[1], Daniel Regan[1], Tony Romeo[2], Theodore Schlegel[3], Jimmy Johnson[1]

[1] *Orthopaedic Bioengineering Research Laboratory, Colorado State University*

[2] *DMG Orthopaedics*

[3] *Hawkins Sports Medicine Research Lab*

Corresponding author's email: vonstade@colostate.edu

Extracellular vesicles (EVs), including exosomes, convey protein and biologic cargo to target cells through receptor mediated interactions and while not well understood, have shown to increase tenocyte proliferation, localization, and downregulate inflammation. Our hypothesis is that tendon derived EVs will also increase wound closure rates. EVs were purified via size exclusion chromatography from primary tenocyte enriched media from ovine infraspinatus tendons harvested 1-2 hours post-mortem and proliferated over 1-3 passages. Similarly, prepared cell lines were used to culture a mono-layer of tenocytes in a 96-well plate. A uniform scratch in the monolayer of each well was formed using the WoundMaker Tool (Incucyte®) and wells were washed with PBS and filled with culture media alone (negative-control), fetal bovine serum (FBS, positive-control) or purified EVs. Images were taken every 6 hours until earliest wound closure to determine the rate of closure via morphometric assessments. There was a significant increase in rate of wound closure when tenocytes received tenocyte-derived EVs up to 53% of the positive- as compared to the negative-control. These data demonstrated that tenocyte derived EVs have the potential to significantly increase tenocyte proliferation and localization leading to increase wound closure in an in vitro model of tendon damage. With the potential for tendon derived EVs to both increase tenocyte bioactivity and to downregulate macrophage pro-inflammatory signaling, these intercellular communicatory packages may contain a balanced means of directing tendon healing. Future investigation into the content of these EVs, their dose relation to effect and in vivo potential is warranted.

Abstract **#1897**

3D FABRICATION OF POROUS HIP IMPLANT TO REDUCE STRESS SHIELDING AND PROMOTE OSSEOINTEGRATION

Seyed Ataollah Naghavi[1], Jia Hua[2,1], Mehran Moazen[3], Steve Taylor[1], Chaozong Liu[1]

[1] *Institute of Orthopaedics & Musculoskeletal Science, University College London, Royal National Orthopaedic Hospital Stanmore, London, HA7 4LP, UK*

[2] *School of Science and Technology, Middlesex University, London, NW4 4BT, UK*

[3] *Department of Mechanical Engineering, University College London, London, WC1E 6BT, UK*

Corresponding author's email: ata.naghavi96@yahoo.com

Total Hip replacement is an effective and successful treatment for osteoarthritis. Currently there are over a million patients which undergo this treatment and the number is expected to double in the next two decades [1]. Patients often undergo revision surgery to replace their hip implant due to either peri-prosthetic fracture or aseptic loosening. Stress-shielding is a mechanical phenomenon that refers to the reduction of bone density as a result of altered stresses acting on the host bone. Current orthopaedic prostheses undergo too much bone resorption secondary to stress shielding due to their solid metallic nature, which are much stiffer than the surrounding bone. With the use of 3D printing technology such as selective laser melting (SLM), it is now possible to produce porous graded microstructure hip stems to mimics the surrounding bone tissue properties [2].

Currently there have been several studies on developing fully porous hip implant, which used different porous structures to reduce stress shielding to some extent (45-50%). This includes structure such as Tetrahedron, Octet and Face and body centered cubic with vertical struts (FBCCZ) [2,3]. However, till now, no study has been performed on the two well known triply periodic minimal surface (TPMS) gyroid and diamond structures. These two TPMS structure have mechanical properties which are very close to cortical bone and are suitable for hip implant application. By using a validated FE model, this research has shown that stress-shielding can be reduced by more than 50% by using graded gyroid structure.

Keywords

Hip Implant; Porous; Stress Shielding

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Abstract **#1898**

3D-PRINTED NANOMATERIAL-EMBEDDED HYDROGEL MATRICES FOR IMPROVED CLINICAL OUTCOMES IN SPINAL SURGERY

Aaqil Rifai[P][1,2], Richard Williams[2]

[1] *School of Engineering, RMIT University, Melbourne, VIC, Australia*

[2] *School of Medicine, Deakin University, Waurn Ponds, VIC, Australia*

Corresponding author's email: aaqil.rifai@rmit.edu.au

Bio-printing is emerging as a novel method to create bespoke items in medicine. Traditionally, bone implants are manufactured using metals, such as titanium and cobalt-chromium alloys.[1] However, the limitations of some of these metals include rapid wear rates and metal ion deposition in the body.[2] To overcome these limitations, a long-term solution is required to promote direct contact between bone and the implant surface and to reduce inflammation. Here, we investigate the ability to 3D-bioprint nanodiamonds (NDs) in a hydrogel matrix to enhance bone growth. Nanodiamond is an ideal candidate for use in biomedicine, due to its excellent biocompatibility.[3] The hydrogel is programmed for passive controlled release of the NDs, meaning that the properties of the NDs can be utilised in a localised setting. The fabricated material complements the natural processes in the body, especially to restore the essential cellular functions of bone. This uniquely 3D-bioprinted material will have a significant impact on the success of spinal implant surgery.

Keywords

3D Bioprinting; Stem Cells

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Abstract **#1899**

DEVELOPING FULLY DEFINED SELF-ASSEMBLY PEPTIDE HYDROGELS FOR HUMAN PLURIPOTENT STEM CELL CULTURE

Farzaneh Ziaee$_P$[1], Eric Du[2], Jingjing Li[1], Jelena Rnjak-Kovacina[1], Pall Thordarson[2], Robert Nordon[1]

[1] Graduate School of Biomedical Engineering, University of New South Wales, Sydney, NSW 2052, Australia

[2] School of Chemistry, The Australian Centre for Nanomedicine and the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, The University of New South Wales, Sydney, NSW, 2052, Australia

Corresponding author's email: f.ziaee@unsw.edu.au

Human pluripotent stem cells (hPSCs) have great potential for tissue engineering and regenerative medicine owing to their unique abilities for self-renewal and differentiation. However, they are cultured on poorly defined, animal-derived extracellular matrix (ECM), not suitable for human use, with batch-to-batch variations. In this study, self-assembled peptide hydrogels which have a capping group that promotes the formation of fibrillar macromolecular complexes, have been developed for culturing HES3 human embryonic stem cells line. These hydrogels mimic the peptide motifs in extracellular matrix proteins that interact with cellular integrin receptors, promoting cell growth and survival. Herein, peptide-gelator-mimics of laminin (Fmoc-YIGSR), collagen (Fmoc-GFFGER), and fibronectin (Fmoc-GFFRGD) were screened for their ability to support the maintenance and growth of human embryonic stem cells and their differentiated progeny. Adhesion, proliferation, migration, colony formation, morphology, and pluripotency were analyzed for single and blended peptide-gelators. The blend Fmoc-GFFRGD + Fmoc-YIGSR (1:1) supported the maintenance and growth of hESCs more compared to other gelators. It is concluded that self-assembled, peptide-hydrogels provide a versatile platform to dissect the role of adhesive motifs for human pluripotent stem cell culture.

Abstract **#1900**

MODULATING PHOTORECEPTOR MATERIAL EXCHANGE WITHIN A HYALURONAN AND METHYLCELLULOSE-BASED HYDROGEL

Margaret Ho$_P$[1], Nicole Yan[2], Arturo Ortín-Martínez[2], Akshay Gurdita[2], Valerie Wallace[2], Molly Shoichet[1,3]

[1] *Institute of Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada*

[2] *Krembil Research Institute, University Health Network, Toronto, Ontario, Canada*

[3] *Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada*

Corresponding author's email: margaret.ho@mail.utoronto.ca

While strategies to transplant photoreceptors to the diseased retina have shown to be a promising approach to treat blindness, cell therapy remains far from clinical translation. It was previously believed that donor cells in the subretinal space integrate and synapse with the host retina to restore vision; however, recent studies have demonstrated that donor photoreceptors rarely integrate and instead transfer cytosolic proteins, including GFP, to host photoreceptors(1,2,3) in a process termed material exchange (ME). This observation suggests that rod-mediated vision rescue is through a mechanism distinct from cell replacement. To exploit ME for therapeutic applications and bridge the gap to clinical translation, it is imperative to investigate the mechanism of ME and identify modulators. Using a flow cytometry-based assay to quantify the transfer of mitochondria, we demonstrate that a hyaluronan and methylcellulose (HAMC) hydrogel(4) reduces the efficiency of photoreceptor ME in vitro. Dissociated retinas from post-natal day 4 mice were stained with MitoTracker Green (MTG) or MitoTracker Red (MTR) and co-cultured in HAMC or standard conditions for 2 days in vitro. There was significant reduction in the proportion of double positive cells (MTR+/MTG+) when retinal dissociates were cultured in the HAMC hydrogel compared to standard 2D culture conditions. This observation is also corroborated in vivo, where we observed a reduction in the efficiency of GFP transfer when donor cells encapsulated in HAMC were transplanted to the recipient retina. The HAMC hydrogel provides a tunable vehicle to add inhibitors or agonists of ME to benefit cell transplantation and future regenerative therapies.

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Abstract **#1901**

IN-VIVO EVALUATION OF 3D PRINTED HYDROGEL WOUND DRESSINGS FOR BURN WOUND HEALING

Fateme Fayyazbakhsh[1], Michelle Amato[1], Michael J. Khayat[2], Amanda Patton[1], Delbert E. Day[2], Ming C. Leu[1]

[1] *Department of Mechanical and Aerospace Engineering, Missouri University of Science and Technology, Rolla, MO 65409, USA*

[2] *Department of Material Science and Engineering, Missouri University of Science and Technology, Rolla, MO 65409, USA*

Corresponding author's email: ffgwb@mst.edu

Burns are hard-to-heal wounds due to the extensive water loss. Hydrogel-based dressings are ideal to provide moist environment and accelerate wound healing. In this research, different bioink formulations based on gelatin and alginate are prepared and evaluated in vitro. After printing the hydrogel mixtures, the printed constructs underwent chemical crosslinking using CaCl2 solution. In order to investigate the structural integrity of the printed constructs, the rheological behavior, tensile strength, and shape fidelity were measured. 3T3 fibroblasts and HaCaT keratinocytes were used to investigate the effect of hydrogel formulation and printing technology on cytotoxicity, and cell adhesion, and dermal/epidermal bilayer formation. Live/dead assay were used to study cell attachment and viability. Lack of amino acids in alginate results in increase in the number of RGD anchors for cell attachment in samples with lower alginate content, while the higher alginate concentration increased the mechanical integrity and printability. To evaluate the in vivo wound healing, animal test was performed on full-thickness burn in rat model with the best sample. After 4 weeks the wound contracture was measured along with further histological analysis using H&E staining. The results of this research highlight the potential of 3D printed hydrogels to induce skin regeneration in full-thickness wounds.

Abstract **#1902**

ELECTROSTATIC-ADSORPTION CONTROLLED RELEASE OF NEUROPROTECTIVE PEPTIDES FOR RECOVERY AFTER ISCHEMIC STROKE

Eric Ho[P][1], Yaoqi Deng[2], Kevin Da[3], Molly Shoichet[1]

[1] *Institute of Biomedical Engineering, University of Toronto*

[2] *Department of Pharmacology and Toxicology, University of Toronto*

[3] *Department of Chemical Engineering, McMaster University*

Corresponding author's email: erics.ho@mail.utoronto.ca

Ischemic stroke affects over 15 million people worldwide every year, and despite significant research patients are faced with limited treatment options. This is due, in part, to the blood brain barrier (BBB). It poses a significant challenge when delivering therapeutics to stroke injured tissue. To address this, our group has developed a non-invasive, localized drug delivery system that circumvents the BBB. The delivery vehicle is comprised of poly(lactic co-glycolic acid) nanoparticles (PLGA NPs) dispersed in a shear-thinning and biodegradable hyaluronan/methylcellulose hydrogel. We have previously shown that proteins can be electrostatically adsorbed to the surface of blank PLGA NPs and can exhibit similar release profiles to conventional encapsulation processes while preserving activity. We hypothesized that this system could be further implemented for the delivery of therapeutic peptides. Release of pituitary adenylate cyclase activating polypeptide (PACAP), a neuroprotective compound that is easily degraded in circulation, was assessed from our vehicle in vitro. Notably, as the zeta potential of the PLGA NPs became more negative and degradation was attenuated, release of PACAP was extended, achieving a burst-free release that was maintained for 14 days. We also demonstrated the bioactivity of released PACAP in the survival of neural cells, and the negligible inflammatory response to the vehicle in cultured astrocytes and microglia. Therefore, we present this drug delivery system as a useful technology for the controlled release of therapeutic peptides for chronic stroke therapy. Future work will investigate the effects of PACAP delivery on neuronal survival and functional recovery in stroke-injured mice.

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Abstract **#1903**

ENZYMATICALLY CROSSLINKED COLLAGEN BIO-INK FOR RAPID AND SUPPORT-FREE HIGH-RESOLUTION BIOPRINTING OF COMPLEX MULTISCALE ORGANS

Dominic RütscheP[1,2]

[1] *1Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zürich, CH-8093 Zürich, Switzerland*

[2] *Tissue Biology Research Unit (TBRU), Department of Surgery, University Children's Hospital*

Corresponding author's email: Dominic.ruetsche@hest.ethz.ch

Collagen is the most abundant structural protein in the human body and constitutes the primary component of the extracellular matrix [1]. Past attempts at bio-printing cellular constructs based on collagen were severely hampered by the slow gelation of the material, resulting in low printing fidelity and resolution. Recent technical advances, such as freeform reversible embedding of suspended hydrogels (FRESH), enabled the printing of more complex architectures. However, the method is still restricted by lengthy pH-driven thermal gelation and the requirement of a support bath[2].

Our group has developed a simple method to modify soluble collagen with an adaptable azide linker, making it susceptible to click-chemistry based bioconjugations. Coupling of this precursor with alkyne terminated enzyme recognition sequences allows for the printing of multidimensional networks due to rapid, bio-orthogonal enzyme-mediated crosslinking (factor XIII, sortase A, HRP) of the extruded bio-ink.

We have demonstrated the multi-scale production of cell-laden human organ scaffolds containing both blood and lymphatic vasculatures. These scaffolds were fabricated using extrusion 3D printing and could be tuned with respect to size and mechanical characteristics. Fast crosslinking kinetics resulted in high printing fidelity even at very low polymer concentrations (<1%). The bio-ink exhibited optimal shear-thinning characteristics and enabled printing of complex hierarchies without supporting materials. The porous microstructure of crosslinked collagen promoted cellular infiltration and the establishment of a microvascular network with different hierarchies necessary to support high cell viability. This novel bio-ink shows great promise as a highly versatile platform material for the bio-printing of large-scale tissues and organs.

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Abstract **#1904**

DEFINING DIFFERENTIATION CONDITIONS OF HUMAN EMBRYONIC STEM CELLS DERIVED CARDIOMYOCYTES WITH ARRAYED CELLULAR MICROENVIRONMENTS

Jake Ireland[P1]

[1] *University of New South Wales*

Corresponding author's email: Jake.ireland@student.unsw.edu.au

Jake Ireland1, Pallavi Srivastava2, Kristopher A. Kilian1, 3

1School of Chemistry, Australian Centre for Nanomedicine, The University of New South Wales, Sydney NSW 2052
2School of Medical Sciences, The University of New South Wales, Sydney NSW 2052
3School of Materials Science and Engineering, The University of New South Wales, Sydney NSW 2052

jake.ireland@student.unsw.edu.au

The optimization of defined differentiation conditions is necessary for the development of the clinical application of human pluripotent stem cell derived cardiomyocytes (hPSC-DCM). Current research has focused on developing defined media formulations for the staged differentiation of hPSC-DCM with little attention to the establishment of defined substrates for hPSC-DCM differentiation and maturation. To address the comprehensive complement of factors that may help regulate hPSC-DCM proliferation, differentiation, and maturation, we report a systematic screening of extracellular matrix proteins (ECMPs) to developed and characterized a defined culture system for the improved differentiation efficiency, sarcomere organization, and calcium handling compared to commercial substrates. Using live-cell calcium imaging to observe the onset of calcium handling in the differentiation process, provides insight into how ECMP substrates can aid and precede the initiation of the first contractions. Observations of sarcomere assembly, alignment, and size also provides evidence of improved maturation on a defined ECMP substrate. In the future, the combination of ECM array techniques with other differentiation stimuli could lay the foundation for a new perspective in the further development of differentiation efficiency and maturation of human pluripotent stem cell derived cardiomyocytes and stem cell research.

Keywords

Human Embryonic Stem Cells; Cardiomyocyte's; Extracellular Matrix

Abstract **#1905**

DOUBLE-LAYERED MICROENCAPSULATED HUMAN HEMATOPOIETIC STEM CELLS FOR DELIVERING PARACRINE FACTORS

Retno Wahyu Nurhayati[P][1], Rafianto Dwi Cahyo[2], Gita Pratama[3,1]

[1] *Universitas Indonesia*

[2] *Osaka University*

[3] *Dr Cipto Mangunkusumo General Hospital*

Corresponding author's email: nretnowahyu@yahoo.com

Transplantation of hematopoietic stem cells (HSCs), progenitors for all types of blood components, is often the last option for patients with certain conditions, e.g. repeated chemotherapy, leukemia, lymphoma, bone marrow disorders. Protocols for isolation, characterization and transplantation have been well established, however, availability of human leucocyte antigens (HLA)-matched donor and scarcity of HSCs are still major challenges for allogeneic transplantation. In the current study, we developed a double-layered microcapsule for delivering paracrine factors from allogeneic HSCs. The umbilical cord blood (UCB) derived-HSCs were entrapped in alginate polymer and double protected by glutaraldehyde cross-linked chitosan coating. A mitochondrial activity assay revealed a restricted cell growth in encapsulated cells. Co-cultures between microencapsulated HSCs with bare HSCs showed significant increases of cell numbers and preserved CD34+ population than monoculture HSCs. This study is the first to report the potency of microencapsulated HSCs to deliver paracrine factors for improving proliferation and HSC maintenance.

Abstract **#1907**

DEVELOPMENT OF 3D PRINTED GRAFTS BASED ON BONE EXTRACELLULAR MATRIX (ECM) FOR BONE REGENERATION

Rotem Hayam$_P$[1], Marcelle Machluf[1]

[1] *Technion- Biotechnology and Food Engineering*

Corresponding author's email: rhayam@campus.technion.ac.il

Bone is a dynamic tissue with the ability to heal and repair without scaring. Nevertheless, in cases of delayed recovery or large non-healing bone defects resulting from trauma, tumor, or infection, bone grafting is required. The extracellular matrix (ECM) naturally provides cells with a supportive framework of structural as well as functional proteins, carbohydrates, and signaling molecules. Furthermore, the ECM was proved to have an important role in successful bone healing, as it increases the interactions among osteoprogenitor cells and growth factors.

The overall goal of the proposed research was, therefore, to reveal the potential of bone ECM (bECM) as a bioactive material for the bioengineering of advanced bone grafts. To this end, ECM was isolated from porcine and human bones, and their distinctive properties were studied, revealing high similarities between the human and the porcine bECM, and indicating the high potential of porcine bECM for bone grafting. Furthermore, in vitro biocompatibility studies showed no indication for an immunological response.

To address the technological aspects of graft additive manufacturing, we first developed a reproducible ECM bio-ink that can be thermally gelled and further crosslinked using natural crosslinkers. Next, we investigated the technological feasibility of 3D-bioprinting complex pbECM scaffolds by addressing the effect of different biomaterial properties and system parameters on the process and its outcomes.

To conclude, we have established the scientific as well as the technological basis for the development of novel 3D-printed pbECM-bone grafts and demonstrated their great potential use for bone regeneration.

Keywords

3D printing; Bone grafts; Bone ECM

Abstract **#1908**

3D VASCULARIZED TISSUE CONSTRUCT USING PROTEIN-BASED COMPOSITES

Linyang Liu[P][1,2], Richard Wang[1,2], Ziyu Wang[1,2], Suzanne M. Mithieux[1,2], Anthony S. Weiss[1,2,3]

[1] *School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales 2006, Australia*

[2] *Charles Perkins Centre, University of Sydney, Sydney, New South Wales 2006, Australia*

[3] *Sydney Nano Institute, University of Sydney, Sydney, New South Wales 2006, Australia*

Corresponding author's email: lliu3358@uni.sydney.edu.au

Vascularization of tissue constructs by incorporating complex vascular networks into three-dimensional (3D) bioengineered constructs to achieve proper oxygenation, nutrient delivery, and waste removal after implantation remains a challenge in tissue engineering. To develop a 3D hybrid vascular system, we embedded tubular tropoelastin and silk fibroin composites (TCs) within enzymatically crosslinked gelatin hydrogel to mimic tissue constructs in customized pulsatile flow-induced bioreactors. The system utilizes alternative protein-based systems. Tropoelastin is highly elastic and cell interactive, promotes endothelial cell attachment and angiogenesis, while silk fibroin confers mechanical strength to the vascular system and bestows the ability to withstand sutures and cope with higher blood pressures. The TCs were fabricated by dip coating on sacrificial ice mold then stabilized. They were subjected to analysis and quality control using techniques including scanning election microscopy, immersed in cell-encapsulated hydrogel which was then cast around the TC to form a hybrid system. Human umbilical vein and other endothelial cells were seeded into the TC statically and dynamically. Concurrently, human cells including dermal fibroblasts were cultured in the hydrogel. Cells in the surrounding matrix, in the vicinity of vessels, were maintained as the porous TC provided proper nutrient diffusion. The 3D vasculature system was tested in a pulsatile bioreactor, which mimicked the native tissue microenvironment and delivered the further benefit of replicating tissue performance. We conclude that this simple and versatile 3D tissue construct system allows for the growth of complex vascularized tissue models in 3D and has great potential for vascularization, for biological and pharmacological uses.

Keywords

Tropoelastin; 3D tissue construct; Bioreactor

Abstract **#1910**

HIGH STRENGTH AND HIGH PLASTICITY NITI-NB POROUS SCAFFOLD FABRICATED BY SELECTIVE LASER MELTING

Yintao Zhang$_P$[1]

[1] *State Key Laboratory of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University*

Corresponding author's email: wang_liqiang@sjtu.edu.cn

For orthopedic implants, it is necessary to have high strength, low elastic modulus, high wear resistance, high corrosion resistance, and good biocompatibility. NiTi shape memory alloy has the above advantages, and the addition of Nb with good biocompatibility can improve its mechanical properties and shape memory effect. To avoid the stress shielding effect, it is necessary to produce a porous structure by additive manufacturing technology to reduce the overall elastic modulus. In this work, gradient porous NiTi-Nb scaffolds were fabricated by SLM, and their mechanical properties and shape memory effect were studied. Firstly, we explored the effect of heat treatment (annealing at different temperatures) on SLMed NiTi-Nb scaffolds' properties and carried out uniaxial compression experiments. It was found that the annealed samples showed high compressive strength, high plasticity, and low elastic modulus. The influence of pore size, gradient interface, and other structural factors on the scaffold's performance was also explored. DSC investigated the samples' shape memory effect, and the microstructure and deformation mechanism was investigated by optical microscope, SEM, and TEM. This work shows that the heat-treated SLMed NiTi-Nb scaffold shows excellent properties of high strength, high plasticity, and low modulus, which has great potential in the application of orthopedic implants.

Abstract **#1912**

ENGINEERING LARGE FUNCTIONAL VASCULARIZED ISLET TISSUES THROUGH MANIPULATING EXTRA REACTION-TIME IN PHOTO-CROSSLINKING PROCESS

Ling Fang[P][1], Ying-Chieh Chen[1]

[1] *Department of Materials Science and Engineering, National Tsing-Hua University, Hsinchu 30013, Taiwan, R. O. C.*

Corresponding author's email: lspss93175@gmail.com

Nowadays, the main approach to tailor mechanical properties of photo-crosslinked gelatin methacrylate (GelMA) is to control the light exposure time to activate free radicals in chain polymerization reaction. The number of activated free radicals increases linearly with light exposure time. Generally, to twice the stiffness of GelMA hydrogel, the exposure time of hydrogel needs to be extended to 3-5 times longer to activate more free radicals.1 However, the more activated radicals, more oxidative stress, and more damage to cells. Here, we developed a new method with less free radicals through executing extra reaction-time right after light exposure instead of stopping the reaction right away to precisely control photo-crosslinking degrees of hydrogel with required storage modulus. Human white adipose tissue derived mesenchymal stem cells (MSCs) and umbilical vein endothelial cells (HUVECs) were encapsulated and patterned into hexagonal three-dimensional (3D) structures to create functional vascular networks in vivo by this method. Our results demonstrated the cell-laden hexagonal structures can guide vasculogenesis and accelerate angiogenesis to form uniformly distributed vascular networks in the thick hydrogel (> 2 mm) within 7 days in diabetic mice with high repeatability and reproducibility. Lastly, the reproducible large-size microvascular networks significantly increase viability and function of transplanted islets in diabetic mice. This developed technique to engineer the large-scale and thick functional vascularized islet tissues will be expected to become the most effective method for clinical use in the near future.

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Abstract **#1914**

EXTRACELLULAR MATRIX SCAFFOLDS DERIVED FROM DIFFERENT MUSCULOSKELETAL TISSUES DRIVE DISTINCT MACROPHAGE PHENOTYPES AND DIRECT TISSUE-SPECIFIC CELLULAR DIFFERENTIATION

Olwyn R Mahon[P][1,2], David C Browe[1], Pierluca Pitacco[1], Pedro Diaz-Payno[1], Aisling Dunne[2], Daniel J Kelly[1]

[1] *Trinity Centre for Biomedical Engineering*

[2] *School of Biochemistry and Immunology, Trinity College Dublin*

Corresponding author's email: olmahon@tcd.ie

The host immune response, specifically macrophage function, is a critical determinant of biomaterial success or failure post-implantation. Extracellular matrix (ECM) derived scaffolds have been shown to promote a pro-regenerative macrophage phenotype and a more constructive remodelling outcome. Here we demonstrate that macrophages adopt distinct phenotypes when exposed to articular cartilage (AC), ligament (LIG) and growth plate (GP)-derived ECM scaffolds. Macrophages were unresponsive to LIG-derived ECM, adopted an M2-like phenotype when exposed to AC-derived ECM, and a hybrid M1-M2 phenotype when exposed to GP-ECM. Furthermore, macrophages expressed higher levels of pro-chondrogenic factors (FGF2), when exposed to AC-ECM, and higher levels of angiogenic and pro-osteogenic factors (VEGF, IL-6 and TNF) when exposed to GP-ECM. In addition, these ECMs can differentially direct the differentiation of skeletal stems, whereby AC-ECM and GP-ECM promote chondrogenic and osteogenic differentiation respectively of multipotent stem/stromal cells (MSCs). In vivo characterization of immune cells following scaffold implantation into a large bone defect demonstrated that AC-ECM drives an M2 macrophage phenotype, while GP-ECM scaffolds promoted a hybrid M1-M2 phenotype and enhanced vascularization and vessel maturation. This distinct response to the implantation of GP-ECM containing scaffolds was associated with increased CD45+ leukocyte and CD3+ T cell infiltration, accompanied by elevated concentrations of IFN-γ and IL-17. Taken together this work demonstrates that the source tissue of ECM scaffolds plays a key role in regulating the phenotype of both macrophages and MSCs. Furthermore, these ECMs can direct the cellular differentiation and production of growth factors essential for the regeneration of their source tissue.

Abstract **#1915**

NUCLEIC- ACID BASED PATHOGEN RECOGNITION RECEPTOR LIGANDS ENHANCE OSTEOGENESIS VIA IMMUNOMODULATION

Paree Khokhani[P][1], Nada R Rahmani[1], Carissa Hing-Wai Wong[2], Keith Yu-Kin Cheng[2], Wing-Hoi Cheung[2], Simon Kwoon-Ho Chow[2], F. Cumhur Öner[1], Rene M. Castelein[1], Saber Amin Yavari[1], Jacqueline Alblas[1], Harrie Weinans[1,3], Moyo C. Kruyt[1], Michiel Croes[1]

[1] *Department of Orthopedics, University Medical Center Utrecht, Utrecht, the Netherlands*

[2] *Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong SAR, China*

[3] *Department of Biomechanical Engineering, Technical University Delft, Delft, The Netherlands*

Corresponding author's email: p.k.khokhani@umcutrecht.nl

Immunomodulation has become an important strategy to advance biomaterials for bone regenerative purposes. Clinically applied therapeutic pathogen recognition receptor (PRR) ligands are known for their ability to skew the immune response in a specific direction. We investigated the effect of various PRR ligands on early inflammatory and bone cell responses. In vitro effects of these ligands on early osteogenic (Day 10) and osteoclast differentiation (Day 6) were assessed by measuring alkaline phosphatase activity in mesenchymal stem cells (n=7) and tartrate-resistant acid phosphate activity in human peripheral blood-derived monocytes (n=4), respectively. We measured TNF-α, IL-6, IL-8, and IL-10 expression in these cells by enzyme-linked immunosorbent assay as an indicator of the ligands' inflammatory properties. To further test their effects in vivo, a closed femoral fracture in osteoporotic rats was performed. The ligands were injected twice locally at the site of fracture. Cytokine production in fracture hematoma (Day 3,n=5) indicated the early inflammatory response. Mechanical testing (Week 4) along with weekly radiographs (n=7) to assess the callus morphometry were used as indicators of bone formation. In vitro, it was found that nucleic acid-based ligands Poly(I:C) and CpG ODN C enhanced early osteogenic differentiation by 4-fold, without inducing exaggerated immune reaction or interfering with osteoclast formation. In vivo, CpG ODN C and Poly(I:C)showed an altered cytokine expression in the hematoma. Qualitative analyses showed differences in calluses among groups. Thus, these findings show that nucleic acid-based ligands have broad osteo-immunomodulatory properties that can be harnessed as a strategy for bone- biomaterials based regeneration.

Abstract **#1916**

GAP JUNCTIONS: THE HOLY GRAIL IN STEM CELL-BASED SUICIDE GENE THERAPY FOR HEAD AND NECK CANCER

Jolien Van den Bosch[P][1], Karen Libberecht[1,2], Tim Vangansewinkel[1,2], Ivo Lambrichts[1], Esther Wolfs[1]

[1] *Faculty of Medicine and Life Sciences, BIOMED, Hasselt University, Diepenbeek, Belgium.*

[2] *Laboratory of Neurobiology, VIB, Center for Brain and Disease Research, Leuven, Belgium.*

Corresponding author's email: jolien.vandenbosch@uhasselt.be

Oral squamous cell carcinomas (OSCC) are malignancies in the oropharynx and the oral cavity. These tumors are often associated with the alcohol and tobacco use as well as HPV infection. Since current treatments cause severe discomfort and significant side effects, the development of an alternative therapy is highly required. This project aims to develop a stem cell-based suicide gene therapy. Hence, human dental pulp stem cells (DPSC) are used as vehicles for gene-directed enzyme prodrug therapy.

Ganciclovir cytotoxicity could be demonstrated successfully in vitro in HSV1-TK expressing DPSC. For successful stem cell-based suicide gene therapy, gap junction formation in DPSC/OSCC co-cultures is essential. Immunocytochemistry indicated the presence of connexin-43 in DPSC/OSCC co-cultures. To assess gap junction functionality, cascade blue was micro-injected in one single cell and dye transfer to neighbouring cells was confirmed. These results suggest gap junctional communication between DPSC and OSCC in a co-culture system. Moreover, these gap junctions are likely to pass cytotoxic ganciclovir from DPSC to OSCC and consequently kill the tumor cells. This will be studied in future experiments.

In conclusion, based on the ganciclovir/HSV1-TK system, gap junction formation is key in the treatment of OSCC using DPSC as vehicles for suicide gene therapy.

Keywords

dental stem cells; suicide gene therapy; head and neck cancer

Abstract **#1917**

NON-VIRAL GENE EDITING APPROACHES TO GENETICALLY AUGMENT AND CORRECT CYSTIC FIBROSIS USING MUCUS PENETRATING PEPTIDE NANOPARTICLES

Aaron M Savage[P][1], Hoda Eltaher[1], Jung Soo Suk[2], James E Dixon[1]

[1] *University of Nottingham*

[2] *Johns Hopkins University*

Corresponding author's email: aaron.savage@nottingham.ac.uk

Cystic fibrosis is one of the most common inherited lethal diseases, caused by mutations in the CFTR gene. Small-molecule therapies have revolutionised patient outcomes but are lifelong interventions and cannot correct all CFTR mutation variants.

We have demonstrated effective non-viral gene delivery to mice using peptide-nanocomplexes composed of plasmid (p)DNA and GET peptides. Glycosaminoglycan (GET) peptides bind and transduce cell membranes and we have generated mucus-penetrating formulations; allowing enhanced delivery and transgene expression to epithelium when aerosolised. Our formulations utilise endosomal-escaping strategies to deliver gene correction/augmentation strategies, presenting a 'genetic cure' which includes treatment of CF patients unaffected by current state-of-the-art therapies.

Our pDNA vector library (lacking CpG dinucleotides, reducing methylation silencing), modified with S/MAR sequences, allows for increases in long-term retention/expression viability. Additional 'integration sequences' have been included, enabling us to target stable integration into the 'safe harbour' AAVS1 locus. We have confirmed our system's efficacy with fluorescent-protein encoding ZsGreen1 and will confirm CF correction in patient-derived lung cells with CFTR transgene pDNAs.

Presently, we are comparing strategies: HDR (homology driven repair) and HITI (homology-independent targeted integration) via CRISPR. We are also exploring directed integration with Rep-mediated (exploiting viral mechanisms targeting P5IEE to the AAVS1 locus) systems and Sleeping beauty transposase.

Here we present the efficiencies and comparison of different systems. Repeat delivery/transfections using GET technology does not affect cell viability, so we can build integration. Ultimately, an aerosol-based strategy to progressively correct CF patients, converting transient gene expression into stable life-long genetic correction, may be viable.

Keywords

Cystic Fibrosis; Gene Therapy

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Abstract **#1918**

SELF-ASSEMBLING PEPTIDE HYDROGEL SCAFFOLDS FOR CORNEAL STROMAL TISSUE ENGINEERING

Ebru Demir$_P$[1], Sevilay Burcu Şahin[1], Sibel Çetinel[1,2]

[1] *Nanotechnology Research and Application Center (SUNUM), Sabanci University*

[2] *Center of Excellence for Functional Surfaces and Interfaces for Nano Diagnostics (EFSUN), Sabanci University*

Corresponding author's email: ebrudem@sabanciuniv.edu

Corneal vision impairment is affecting 4.9 million people, globally. Treatment options to repair damaged corneal tissue is limited to corneal transplantation and only a limited number of people can receive corneas while millions being in the waiting list due to the limited tissue availability. An artificial cornea would provide an alternative solution for transplantable tissue shortage. An ideal bioengineered corneal scaffold for vision recovery is expected to provide optically transparency, biocompatibility, triggering tissue healing, and cornea-like structure. Various biomaterials including decellularized scaffolds, natural polymers and peptide amphiphiles have been found promising for corneal applications due to their biocompatible, permeable and transparent nature.

Hydrogels with their high-water content are promising candidates for 3D cell culture for corneal stromal cells. Here, we aim to utilize self-assembling peptide hydrogels as stromal substitutes due to their ease of synthesis and gelation properties. Among self-assembling peptides, β-sheet forming peptides can easily be triggered to form hydrogel network by adjusting the ionic strength of the solution. In this study, MAX8, a 20-residue self-assembly peptide, was folded and formed a hydrogel within serum free corneal keratocyte cell culture media. MAX8 hydrogels were produced with three different biomarker peptides to provide cell adhesion and anti-angiogenic properties while preserving transmittance and mechanical stability to mimic the cornea structure. The hydrogels showed advanced biocompatibility to promote cell adhesion and proliferation for corneal keratocytes. ECM production of keratocytes within peptide hydrogels and tissue specific properties such as the optical transparency and mechanical strength of produced 3D tissue substitutes are being evaluated.

Keywords

Cornea; 3D peptide hydrogel; Stromal keratocyte

Abstract **#1919**

PRIMARY CORNEAL CELL CULTURES ON 3D PEPTIDE HYDROGEL / ELASTOMER MEMBRANE SCAFFOLDS AS CORNEAL TISSUE SUBSTITUTES

Sevilay Burcu Şahinᵽ[1], Ebru Demir[1], Kamal Asadipakdel[1], Sibel Çetinel[1,2]

[1] *Nanotechnology Research and Application Center (SUNUM), Sabanci University*

[2] *Center of Excellence for Functional Surfaces and Interfaces for Nano Diagnostics (EFSUN), Sabanci University*

Corresponding author's email: sevilay.sahin@sabanciuniv.edu

Cornea is the outermost layer of the eye that provides protection as well as the first line of light refraction for proper vision. Cornea is a transparent tissue composed of highly ordered collagen fibrils and providing pressure resistance. All three corneal cell types have unique properties to constitute this transparent, permeable and durable tissue. Corneal endothelial cells residing on Descement's membrane restrain fluid to the cornea. Corneal stromal cells, keratocytes, give the unique structure of the stroma play an important role in light transmission. Epithelial cells provide the protective barrier, light reflection, water, gas, and nutrient transport in and out of stroma. External chemical/mechanical damage, aging and diseases can directly affect these cells and thereby cause visual disturbances. Currently, the only effective treatment for corneal vision impairment is keratoplasty, namely corneal transplantation. However, there is a limited number of donor cornea. A bioengineered cornea as a tissue substitution holds the promise to overcome transplantable tissue shortage as well as drawbacks related to implant quality.

Here we aim to engineer a β-sheet peptide hydrogel supported with an elastomer layer for increased durability, controlled shape and surface topology. We, initially isolated primary human corneal cells. Epithelial and endothelial cells were cultured on elastomer membranes as mimics of Descemet's membrane and Bowman' layer of natural cornea. As a stromal layer mimic, keratocytes were cultured on 3D peptide hydrogels. Hydrogels and elastomer membranes were transparent and non-toxic for the cells. Proper ECM production, optical transparency and mechanical strength of tissue substitutes are under investigation.

Keywords

Cornea; 3D peptide hydrogel; Elastomer membrane scaffold

Abstract **#1920**

PREPARATION OF DRUG-LOADED NANOPARTICLES AND THEIR INCORPORATION INTO THERMO-RESPONSIVE HYDROGELS AS INJECTABLE DRUG DELIVERY SYSTEMS

Elif Gülin Ertuğral[P][1], Özgül Gök[1]
[1] *Department of Medical Engineering, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey*
Corresponding author's email: elifgulinertugral@yahoo.com

INTRODUCTION: Drug delivery systems allow the therapeutic substances to be administered to a specific part of the body. For this purpose, biomaterial-based platforms have been designed that can release the drug active ingredient it contains. Herein, we develop a minimally invasive drug delivery system that is targeted only to ovarian cells and has the potential to eliminate inflammation in the operated area after any surgical intervention in the ovaries and reduce ovarian cell loss/damage.

METHOD: An anti-inflammatory drug curcumin was loaded with 95% efficiency into chitosan-coated sodium alginate nanoparticles and then functionalized with folic acid for providing targeting property to nanocarrier towards ovarian cells. After their characterization with FT-IR, UV-Vis spectrophotometer and TEM, they were incorporated into thermo-sensitive pNIPAm–based hydrogels as injectable drug delivery system. Rheological tests, swelling capacity, morphological analysis and evaluation of thermo-responsiveness of hydrogels were evaluated.

RESULTS: This polymeric scaffold design was demonstrated to form the hydrogel structure mixed with nanoparticles to gel in a very short time when injected into the tissue wound region at body temperature.

DISCUSSION AND CONCLUSION:
The obtained multifunctional scaffold was shown to liberate drug molecules 3 times slower compared to intact free nanoparticles, which might contribute to local administration of therapeutic agents during minimally invasive tumor resection based surgeries for both facilitating wound healing and sustained diminishing of chronic inflammatory immune system responses.

ACKNOWLEDGEMENTS:
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Keywords
Anti-inflammatory; Injectable Hydrogels; Curcumin

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Abstract **#1921**

DEVELOPMENT OF A NEXT GENERATION, NATURAL POLYMER BASED, BIOARTIFICIAL PANCREAS FOR THE TREATMENT OF TYPE 1 DIABETES

Jonathan D Hinchliffe[P][1], MoyinOluwa Odugbemi[2], Ipsita Roy[3], Nigel Irwin[4], Victor Gault[5]

[1] *The University of Sheffield*

[2] *University Of Westminster*

[3] *University of Sheffield*

[4] *Ulster University*

[5] *Ulster University*

Corresponding author's email: jhinchliffe3@sheffield.ac.uk

Type 1 diabetes is described as an autoimmune condition which, through pathological immune system action, enables destruction of the β-cells of the pancreatic islets of Langerhans, reducing insulin production capacity (1). A proposed biomaterial solution to this problem has been microcapsule immunoisolation, where the (often alginate) microspheres of islet containing membranes separate the immune system from implanted allogeneic and xenogeneic tissue (2), However, barriers to effective therapies remain, including hypoxia prior to revascularization and microbead mechanical degradation (3).

This work builds on previous research exploring the use of biodegradable, non-immunogenic Polyhydroxyalkanoate (PHA) polymers for the generation of a semi artificial pancreas (4). Pseudomonas Putida fermentation was used to produce a PHA which was purified and described as Poly(3-hydroxyoctanoate)co(3-hydroxydecanoate) (PHOHD). The viability and glucose dependent insulin secretion of BRINBD11 and INS-1 832/13 cells (a pancreatic cell line) grown on PHOHD was compared to cells grown on poly(Caprolactone) and poly(3-hydroxybutanoate) films. Following this, the islet cell lines were encapsulated in calcium/strontium-crosslinked alginate and bioprinted into sub mm wells within a novel 3D-printed PHOHD construct design. Islet cell lines were described in terms of viability, glucose responsiveness and insulin secretion. This work will act as a template for a construct encapsulating whole human islets and modifications to both the alginate and the PHOHD components to improve mechanical properties, glucose responsiveness, vascular tissue ingrowth, oxygen availability, non-immunogenicity, and antibacterial capacity.

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Abstract **#1922**

DEVELOPMENT OF ANTIMICROBIAL CATHETER VIA NOVEL SURFACE CONJUGATION STRATEGIES

İrem Soyhan$_P$[1], Özgül Gök[2], Nihan Ünübol[3]

[1] *Department of Medical Biotechnology, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey*

[2] *Department of Medical Engineering, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey*

[3] *Acibadem University*

Corresponding author's email: iremsoyhan@gmail.com

INTRODUCTION: Today, 40% of the hospital infections are caused by the urinary tract infections and within this; 80% of them are due to the catheters. Especially in the cases which the catheter usage is obligatory more than one week, microorganisms can produce biofilm on the surface of the catheter [1,2]. Hence, the main goal is the obtain more efficient catheter than already existing catheters with antimicrobial properties through a surface modification with a higher binding efficiency using a single intermediate binding molecule.

METHOD: The thiolated antimicrobial peptides(C-AMPs) were designed in the scope of the project and chemically synthesized, purified and characterized with LC-MS/MS systems. Antimicrobial effect examined with in vitro cytotoxicity, minimal inhibitory concentration, and hemolytic assays. With a single intermediate molecule, antimicrobial peptides were immobilized to silicone catheter surface through thiol-ene chemistry. Chemical, physical characterizations of catheter surface were done, stability of the immobilization examined.

RESULTS: For the EDS point analysis carbon (C), oxygen (O), nitrogen (N), and sulfur (S) atoms were chosen and especially the presence of S indicates the presence of C-AMPs and their immobilization to the surface

DISCUSSION AND CONCLUSION:
This result indicates that C-AMPs could be immobilized to silicone surfaces via a single intermediate molecule rather using double linker similar in literature to allow us to generate antimicrobial medical implants.

ACKNOWLEDGEMENTS:
This research has been supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK Project No: 118Z859).

Keywords
Antimicrobial Peptides ; Silicone Catheter Surface Modification; Surface Characterization

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Abstract **#1924**

FUNDAMENTAL STUDY OF DECELLULARIZATION METHOD BY SUPERCOOLING AND HIGH HYDROSTATIC PRESSURE

Daiki Zemmyo$_P$[1], Masashi Yamamoto[1], Kiyoaki Sudo[1], Shogo Miyata[2]

[1] *Graduate School of Science and Technology, Keio University*

[2] *Department of Mechanical Engineering, Faculty of Science and Technology, Keio University*

Corresponding author's email: nekonigohan@keio.jp

High hydrostatic pressure (HHP) can be applied to large three-dimensional tissues for decellularization without the risk of residual reagent. However, high level of HHP denature extracellular matrix (ECM), whereas low level of HHP remove cells not-sufficiently. Supercooling is the liquid state below the freezing point, and does not cause physical damage. By preserving the tissue in a supercooled state without a cryoprotectant, it is considered that cells are damaged due to osmotic pressure between intracellular and extracellular fields whereas the structure of ECM is retained. In this research, the effect of combined application of supercooling and HHP on decellularization was investigated. Specifically, the device to enable supercooling preservation of cells and cell-containing constructs and the device that could apply HHP were developed. Human skin fibroblasts (NB1RGB) were preserved at 4, -4, or -8 ℃ in nonfreezing state for 12 h, then 100, 150, and 200 MPa of HHP were applied on the cells. After HHP application, cell morphology, cell viability, cell adhesion, and cell proliferation were evaluated. As a result, denaturation of cell membrane was increased with decrease in storage temperature. In addition, viability, adhesion, and proliferation of cells subjected to lower storage temperature decreased when the cells were treated with the same level of HHP. In conclusion, it was suggested that decellularization could be promoted at a lower level of HHP than that of previous approaches by combined effect of supercooling and HHP.

Keywords

Supercooling; High Hydrostatic pressure; Decellularization

Abstract **#1925**

FORCE-MEDIATED MOLECULE RELEASING TOUGH HYDROGEL BIOMATERIAL FOR TISSUE ENGINEERING APPLICATIONS

Honda Jayathilaka$_P$[1], Thomas Molley[2], Yuwan Haung[3], Shariful Islam[2], Meredith Silberstein[4], Jamie Kruzic[2,3], Kristopher Kilian[1,2]

[1] *School of Chemistry, University of New South Wales (UNSW Sydney), Sydney NSW 2052, Australia*

[2] *School of Materials Science, University of New South Wales (UNSW Sydney), Sydney NSW 2052, Australia*

[3] *School of Mechanical Engineering, University of New South Wales (UNSW Sydney), Sydney NSW 2052, Australia*

[4] *Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA*

Corresponding author's email: z5217432@ad.unsw.edu.au

Fores play a crucial role in our bodies. The forces generated in the extracellular matrix can direct the release of growth factors to cells in the surrounding tissues, which catalyzes important bioactivities such as growth factor release or immobilization[1]. The extracellular matrix's biopolymers are evolved to support these complex functional dynamics. Therefore, the extracellular matrix is described as a complex hydrogel & natural mechanochemical model.

We have designed a force-responsive double-network hydrogel material to recapitulate the native extracellular matrix's force medicate molecule release. It releases tethered molecular cargo in response to tension and compression under physiological forces. The flex-activated force-sensitive mechanophore crosslinkers give the hydrogel network a dynamic nature and maintain the overall gel architecture without degradation during successive molecule release steps. The results showed that the gel with 5 wt% mechanophores releases 10%, 15%, 20%, and 7% of molecules under compression of 0.1 MPa, 0.5 MPa, 1 MPa, and 2 MPa, respectively. This work may pave the way to develop force activated biomaterial such as implant coatings, "smart" bandages, contact lenses, and soft robotics for advanced tissue engineering applications.

Keywords

Mechophore; Tough hydrogel; Biomaterial

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Abstract **#1926**

THERE'S MORE TO FAT THAN STEM CELLS, CHARACTERISING FAT GRAFT MATERIALS TO UNDERSTAND THE REGENERATIVE PROPERTIES OF ADIPOSE TISSUES

Victoria L Workman[P][1], Samuel J Higginbotham[1], Sabiniano Roman[1], Alejandra Alvarez Penuelas[1], Victoria Giblin[2], Sheila MacNeil[3], Nicola Green[3], Vanessa Hearnden[3]

[1] *Department of Materials Science and Engineering, The University of Sheffield.*

[2] *Department of Plastic Surgery, Sheffield Teaching Hospitals NHS Trust, Sheffield.*

[3] *Department of Materials Science and Engineering, The University of Sheffield*

Corresponding author's email: v.workman@sheffield.ac.uk

The discovery of adipose derived stromal cells (ADSCs) in 2001 led to an explosion of studies investigating their potential in a range of clinical applications. In parallel, plastic surgeons developed novel techniques following observations that fat grafting can not only restore volume but can also improve tissue repair and regeneration (1.). At present we have limited understanding of how adipose tissue processing affects biological function of injected tissues, which is urgently needed to optimise clinical fat grafting techniques. This study aimed to fully characterise lipoaspirate and formulations of adipose tissues (FAT); bridging the gap between in vitro ADSC studies and clinical fat grafting results.

Adipose tissue collected from consenting patients (NHS ethics 15/YH/0177) was processed to produce four different FAT, namely: lipoaspirate, emulsified fat (or "nanofat"), lipocondensate (condensed SVF, with lipid removed) and cultured ADSCs. We observed lipoaspirate, emulsified fat and lipocondensate all retained some of the microstructure of adipose tissues with intact adipocytes and evidence of blood vessels. Via cytokine array analysis, adipose tissues secreted more chemokine signals compared to ADSCs while all samples secreted factors involved in extracellular matrix regulation. Functional assays demonstrated conditioned medium from adipose tissue but not cultured ADSCs were able to inhibit myofibroblast differentiation.

This study provides important data on the composition and secretory phenotype of FAT and highlights important differences between cultured ADSCs and whole tissue. It is evident the regenerative effects observed from fat grafting arise from heterogeneous tissues rather than ADSCs alone, with work ongoing to understand the mode of action.

Keywords

Liposuction

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Abstract **#1927**

ON-CHIP 3D CELL CULTURE PLATFORM FOR TUMOR MODELING AND DRUG SCREENING

Özüm Yıldırım[P][1], Ahu Arslan Yıldız[1]

[1] *İzmir Institute of Technology, Faculty of Engineering, Department of Bioengineering, İzmir, Turkey*

Corresponding author's email: ozumyildirim@iyte.edu.tr

Three-dimensional (3D) cell culture allows cell-cell and cell-matrix interactions and provides more in vivo like models rather than 2D cell culture which cannot fully mimic native tissue. 3D cell culture on microfluidics allows formation of 3D structures that mimic the physiological and chemical microenvironment for cells[1]. These microfluidic platforms also downsize bench-top laboratory to a microchip, require miniaturized reagent, and are convenient for dynamic drug screening[2]. In this study, a microfluidic platform was designed which is housing a PLLCL scaffold fabricated by electrospinning methodology. The electrospun material resembles fibrillar structure of the extracellular matrix while favoring cell adhesion and proliferation with the help of micro-to nano-meter range fibers[3]. By combining these advantages of electrospinning technique with microfluidic system, here it was aimed to develop scaffold-based 3D tumor models and to provide a platform where drug-screening applications can be carried out easily and in a controlled manner. Developed platform has a reservoir, which allows the fabrication of free-standing electrospun PLLCL scaffold directly on top of the microfluidic chip, a channel for transporting of cell, media, and drugs, an inlet and outlet to be applicable for both static and dynamic culture. Following characterization of scaffold and microchip, 3D tumor formation was achieved on this platform by using HeLa cells, which results in high cell viability and proliferation rate. Drug screening analysis provided more in-vivo like results. These results demonstrate that this developed platform is convenient for formation of various 3D tumor models and a potential candidate for drug screening and toxicity analysis.

Keywords

3D cell culture ; 3D tumor-on-a-chip ; Drug screening

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Abstract **#1929**

MICROFLUIDIC 3D HMSC POTENCY ASSAY TO RECAPITULATE COMPLEX PROTEIN REGULATION AND FEEDBACK PROCESSES OF IN VIVO PHENOTYPE

Rebecca S Schneider$_P$[1], Evelyn Kendall Williams[1,2,3], Alexandra C Vela[1], Andrés J García[1], Wilbur A Lam[1,2,3]

[1] *Georgia Institute of Tech, Atlanta GA, USA*

[2] *Emory University School of Medicine, Atlanta GA, USA*

[3] *Aflac Cancer and Blood Disorders Center of Children's Healthcare of Atlanta, Atlanta GA, USA*

Corresponding author's email: rebecca.schneider@gatech.edu

Human mesenchymal stromal cells (hMSCs) have long been identified for their beneficial anti-inflammatory and immunomodulatory properties making them a promising cellular therapy candidate for a wide variety of disease indications. Despite almost three decades of clinical development, few hMSC products have had success making it to market.[1] While early phase hMSC clinical research progressed rapidly with promising early results, significant setbacks were faced at advanced clinical phases as clinical endpoints failed to be met.[2–6] These late stage set backs are largely attributed to the inability to accurately assess cell potency through scale up processes and across variable donor populations.[6] To address these challenges, we have engineered a perfusable microfluidic platform as a tissue-on-a-chip to predict hMSC functional performance. The system we have engineered encapsulates hMSCs in 4-arm poly(ethylene glycol)-maleimide (PEG-4MAL) hydrogel environments within a perfusable microfluidic device. We have previously demonstrated that the immunomodulatory secretion profile of hMSCs is significantly influenced by their microenvironment.[7] We have found a loss of functional utility of established potency markers IDO and PD-L1 of hMSC cultured in our microfluidic system compared to traditional 2-dimentional static culture, as well as differential protein regulation between the two platforms. Furthermore, when compared to an in vivo model, we found that the protein correlations of specific immune-relevant pathways are conserved in our microfluidic system but not 2D cultures. From these findings, we will establish a more predictive and robust hMSC potency metric.

Keywords

microfluidic ; potency

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Abstract **#1930**

GENERATION OF EXTRACELLULAR MATRIX BIOMATERIAL DERIVED FROM DECELLULARIZED SHEEP VEIN TISSUE FOR TISSUE ENGINEERING APPLICATIONS

Essi M. Niemi[1,2,3], Maria Ekman Stensland[4], Sachin Singh[4], Tuula Nyman[4], Håvard Attramadal[2], Antonio Rosales[1,2], Jonny Hisdal[1,2], Hanne Scholz[2,3,5]

[1] *Department of Vascular Surgery, Oslo University Hospital, Oslo, Norway*

[2] *Institute for Surgical Research, Oslo University Hospital, Oslo, Norway*

[3] *Hybrid Technology Hub, Centre of Excellence, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway*

[4] *Department of Immunology, University of Oslo and Oslo University Hospital, Oslo, Norway*

[5] *Department of Transplant Medicine, Oslo University Hospital, Oslo, Norway*

Corresponding author's email: essi.niemi@rr-research.no

Decellularized extracellular matrix (ECM) is an attractive and natural biomaterial for tissue engineering applications due to the native biochemical cues and composition. Vasculature is the key to viable tissues, which is still the greatest challenge in the field of regenerative medicine for building larger tissues and organs. Manufacturing ECM derived biomaterials from native vessels could be a possible solution to generate artificial vasculature.

We therefore developed a simple but efficient composition preserving decellularization method for sheep vein tissue to produce vascular extracellular matrix hydrogels for tissue engineering applications such as 3D bioprinting. The sheep veins for the study were harvested from local university farm, and frozen to -80 °C as first step of the decellularization, followed by CHAPS detergent and Benzonase nuclease washes. The PicoGreen assay was used to measure the DNA content, and collagen and glycosaminoglycan (GAG) contents were detected by hydroxyproline and Blyscan assays. The vein tissue proteome was assessed by liquid chromatography mass spectrometry (LC/MS).

The decellularized veins had less than 1 ng mg-1 total DNA per dry weight (mean 99,6 % reduction, n=6) while total collagen and GAGs were highly preserved compared to untreated native control. These results and absence of nuclei were also confirmed by histological analysis. The proteomic profiling showed overall well-preserved ECM, suitable for biocompatibility determination of the material for further tissue engineering applications.

These preliminary results are strong for further biomaterial development and aimed to be translated to human material from cardiac bypass surplus tissues for allogeneic option for vascular tissue engineering.

Keywords

Decellularization; ECM hydrogels; Vascular tissue engineering

Abstract **#1931**

DESIGN OF A NOVEL BIOINK SUITABLE FOR THE 3D PRINTING OF LYMPHOID CELLS

Davide Ribezzi[P][1], Federica Barbaglio[1], Riccardo Pinos[1], Lorenzo Bonetti[2], Silvia Farè[2], Cristina Scielzo[1]

[1] *Unit of Malignant B Cell Biology and 3D Modeling, Division of Experimental Oncology, IRCCS Ospedale San Raffaele, Milano, Italy*

[2] *Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Milano, Italy*

Corresponding author's email: ribezzidavide@gmail.com

For decades in vitro 2D cell culture techniques have been employed in research, but they fail to recapitulate the complexity of natural tissues. 3D Bioprinting could potentially overcome this drawback due to the possibility to control the geometry and the 3D disposition of living cells within the scaffold.

This study describes a procedure to design and characterize a novel bioink for extrusion-bioprinting, analysing different blend formulations of alginate, gelatin and methylcellulose, suitable to bioprint and study lymphoid cells, in particular Chronic Lymphocytic Leukemia (CLL) cells. Rheological properties, the printability and the sol-gel transition temperature of the formulations were investigated to define the optimal printing parameters. Stability under culture conditions of printed scaffolds was investigated and compression tests were performed on bioprinted scaffolds to compare their mechanical properties to a fresh lymphoid tissue's ones. Finally, MEC1, a CLL cell line, was bioprinted to analyse cell viability, cell density and cell tendency to leave the scaffold over time.

Results showed that, for the selected blends, good shape fidelity and printing accuracy are achieved with a limitation on the scaffold height. Scaffolds have withstood to culture conditions showing stability up to three weeks and their mechanical properties were similar to the ones of tonsillar tissue and to the ones reported in literature [1]. High cell viability after 21 days was observed also bioprinting MEC1 and lymphoid stromal cells, suggesting the possibility to use this material to mimic CLL cells microenvironment.

Keywords

Bioprinting; Hydrogels; Leukemia

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Abstract **#1932**

DEVELOPMENT OF AN ADVANCED LIVER ORGAN-ON-CHIP INTEGRATING HYDROGEL MIMICKING CELL MATRIX

Taha Messelmani$_P$[1], Zied souguir[1], Victoria Maes[1,2], Anne Le Goff[1], Eric Leclerc[1], Elodie Vandenhaute[2], Cécile Legallais[1], Nathalie Maubon[2], Rachid Jellali[1]

[1] *Université de technologie de Compiègne, CNRS, Biomechanics and Bioengineering, Centre de recherche Royallieu-CS 60319 -60203 Compiègne Cedex*

[2] *HCS Pharma, 250 rue Salvador Allende, Biocentre Fleming Bâtiment A, 59120 Loos, France*

Corresponding author's email: messlmani.taha@gmail.com

The 3R guidelines impose the replacement of animal testing with alternative models. Liver-on-chip represents a promising alternative for drug screening and toxicological assays. However, optimisation is required in order to mimic in a trustworthy way the functionalities and the microsomal environment of liver cells. In this work, we developed a new liver-on-chip model including a relevant 3D matrix to support hepatic cell growth and function. This 3D matrix (BIOMIMESYS® Liver hydroscaffold) is a porous hydrogel composed of liver ECM components. The HepG2/C3a cell line was cultured in the gel, either on-chip under flow for 20 days or in static condition in 96-well plates. In contact with the hydrogel, cells tend to assemble and form spheroids while attaching to the adhesion sites of the hydroscaffold. First of all, flow rate measurements indicate that an optimal flow is preserved with and without the gel. An increase of pressure in the biochip has been noted due to cell growth which caused an increase of the hydraulic resistance. Cell viability, physiology and spheroid integrity were studied by immunostaining of the tight junctions and the development of the actin network, in addition to the albumin production and compared in the two conditions. Dynamic conditions were associated with a significant increase in albumin production, a higher viability and a conservation of the integrity of the spheroids compared to static conditions. These results highlight the tremendous role of the liver-on-chip model coupled with ECM matrix in a dynamic condition in the enhancement of cell functions and physiology.

Abstract **#1933**

INFLUENCE OF MN DOPED CALCIUM PHOSPHATE 3D PRINTED SCAFFOLDS FOR OSTEOCHONDRAL TISSUE REGENERATION

Meenakshi Kamaraj$_P$[1], Uday Kiron Roopavath[1], Subha Narayan Rath[1]

[1] *Indian Institute of Technology Hyderabad, India*

Corresponding author's email: bm18resch01004@iith.ac.in

Osteochondral regeneration remains a vital problem in osteoarthritis due to the cartilage defect site's low healing mechanism. Articular defects are challenging to treat because they extend deep to the subchondral bone range, and those two tissues are dissimilar. Surgical therapies have been advocated to treat diseased site likely micro fracturing, autologous chondrocyte implantation and autograft. These treatment methods have limitations such as fibrocartilage formation, donor site morbidity, the mismatch between tissue repaired site and lack of tissue integration. To improve the tissue repair biomaterials widely used but still could not mimic the naïve tissue structure. 3D printing gained attention for developing 3D scaffolds that can provide mechanical strength and allows tissue regeneration. In this context, using calcium-deficient apatite (CaP) with Mn for the osteochondral repair has been shown to improve the tissue regeneration. The influence of Manganese (Mn) with CaP on cell behaviour will tend to evoke human mesenchymal stem cell (hUMSCs) differentiation. The 3D printed Mn-doped CaP scaffolds were studied for in vitro cell proliferation and differentiation of human mesenchymal stem cells (UMSCs). The structure morphology, and extruded line width has been checked using scanning micrographs. Increasing Mn concentration led to increased cell attachment on the 3D printed scaffolds. The fabricated constructs analyzed for bone-specific alkaline phosphatase (ALP) marker using ALP staining and assay. All the group shows the positive expression of ALP on day 14. The Mn-CaP composition revealed the best set of bioactivity properties, potentially a good candidate for future applications of CaP materials in osteochondral regeneration.

Keywords

3D Printing; Osteochondral ; Manganese

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Abstract **#1934**

MAKING IT STICK: A NOVEL APPROACH TO BIND AND PRESENT PHYSIOLOGICAL AMOUNTS OF BMP-2 VIA COLLAGEN-BASED SUBSTRATES

Stylianos Sarrigiannidis[P][1], Oana Dobre[1], Matthew Dalby[1], Manuel Salmeron-Sanchez[1]

[1] *University of Glasgow*

Corresponding author's email: s.sarrigiannidis.1@research.gla.ac.uk

Introduction: Collagen type I, often used in tissue engineering, does not have a high affinity to GFs such as BMP-2. Absorbable collagen sponges loaded with BMP-2, even the medically approved systems such as Infuse®, use high concentrations of BMP-2 [1] to compensate for the swift BMP-2 leakage out of the material. High concentrations of BMP-2 can have adverse side effects e.g. ectopic bone formation [2]. The aim of this study is to use a new peptide to bind low amounts of BMP-2 onto collagen substrates. This allows controlled, local BMP-2 presentation to the biological environment to promote osteogenesis.

Methods: A protein fragment linking the fibronectin collagen binding and GF binding domains was expressed in E. Coli and incorporated into collagen substrates in 2D, 2.5D and 3D. The BMP-2 release from collagen combined with the peptide was assessed. Human mesenchymal stem cells (hMSCs) were seeded on the material to conduct viability, attachment and osteogenic differentiation.

Results: The new fragment successfully binds to collagen substrates and allows for the sustained release of BMP-2. Osteogenic differentiation experiments show that the collagen substrates treated with the peptide and BMP-2 show upregulation of osteopontin (OPN) - 21 days as well as calcium deposition after 24 days.

Conclusion: This system is an innovative method of absorbing and retaining physiological amounts of BMP-2 to promote osteogenic differentiation of hMSCs. It has the potential to improve already medically approved collagen-BMP-2 materials and can be expanded to other GFs in the future for tissue regeneration.

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Abstract **#1935**

3D BIOPRINTED GELMA VASCULAR CONSTRUCTS AND MATURATION USING BIOREACTOR SYSTEM: VASCULAR TISSUE REGENERATION

Meenakshi Kamarajp[1], Sushma Priya Singh[1], Subha Narayan Rath[1]

[1] *Indian Institute of Technology Hyderabad, India*

Corresponding author's email: bm18resch01004@iith.ac.in

Vascular tissue engineering requires the implants which avert the restenosis, thrombosis and immunological reactions and must withstand physiological pressures without any leakage. In the clinic, synthetic polymer vascular grafts such as PTFE (Teflon) and PET (Dacron) have improved the diseased vessel function. Even though synthetic grafts provide good mechanical properties, it could not mimic the native blood vessel. Hydrogels gained attention to fabricate the 3-dimensional (3D) scaffolds. The existing casting, salt leaching methods have limitations in developing a biological scaffold. To improve the fabricating methods 3D printing procured attention for fabricating cells encapsulated scaffolds by layer-by-layer deposition in a controlled architecture. In order to use hydrogel for 3D bioprinting, we opted for photo-cross-linkable gelatin methacrylate (GelMA) due to its better biocompatibility and printability. We have performed a pilot study on the 3D bioprinted scaffolds with human mesenchymal stem cells (MSCs) and endothelial cells (ECs), namely cell viability by live/dead staining and cell proliferation using Alamar blue assay. From the staining results, we could observe that cells were attached and have actin filament spreading when compared with other cells encapsulated scaffolds (Alginate-gelatin). Additionally, we aimed to mimic a blood vessel's physiological conditioning using bioreactor (dynamic culture condition). The flow-induced physiologic system plays a pivotal role in cell alignment and vessels maturation. This study explores mechanical stimulation's influence on the 3D bioprinted scaffolds by a bioreactor system that could contribute to cell alignment for vessel maturation.

Keywords

3D Bioprinting; Vascular tissue regeneration; Bioreactor

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Abstract **#1936**

3D BIOPRINTING OF VASCULAR NETWORKS IN CELL-LADEN HYDROGEL CONSTRUCTS

Lorenzo Bova[P][1,2], Dario Falcone[1], Aaron Kavanaugh[2], Sara Micheli[1], Luca Zanella[1], Paul Benya[2], Fabrizio Billi[2], Elisa Cimetta[1,3]

[1] *University of Padua, Department of Industrial Engineering (DII) - Via Marzolo 9, 35131 Padova, Italy.*

[2] *UCLA Department of Orthopaedic Surgery, David Geffen School of Medicine, Los Angeles, California, USA.*

[3] *Fondazione Istituto di Ricerca Pediatrica Città della Speranza (IRP) - Corso Stati Uniti 4, 35127 Padova, Italy.*

Corresponding author's email: lorenzo.bova@phd.unipd.it

Advances in 3D bioprinting technology enabled fabrication of thick in vitro tissue models, further pushing the need for a supporting vascular network [1, 2], ideally built with simple and versatile approaches to create user-tunable structures. Our strategy involved the combined use of Gelatin Methacrylate (GelMA) and Pluronic F127 (PLU) hydrogels. GelMA is known for its many applications in tissue engineering [3], and to optimize its performance as a cell-laden hydrogel we blended it with PLU (0.1 to 1%). The addition of PLU, which is washed away after GelMA crosslinking, led to an increased porosity with a bimodal distribution of radiuses (10-20 µm and 50-100 µm) without affecting the bioink printability. Our second hydrogel is a high-concentration Pluronic F127 (PLU) solution that can be printed in pillars and short bridges without collapsing, enabling printing both 2D and 3D freestanding filament networks. The stepwise 3D printing protocol is as follows: it started with the deposition and partial crosslinking of a thin layer of a cell-laden photocurable GelMA-PLU blend, followed by the construction of the PLU channel networks on top of it; these networks were then surrounded by more cell-laden hydrogel, finally photo-crosslinked to yield the final construct. After washing away the sacrificial PLU, we seeded the hollow channels with HUVECs to cover the internal vasculature surface with an endothelial monolayer. When using Mesenchymal Stem Cells (MSCs) in the hydrogel bulk, the final structure and porosity of the material not only enhanced cell adhesion and viability, but also increased MSCs osteogenic potential.

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Abstract **#1937**

THE IMPACT OF ADIPOSE TISSUE HARVESTING SITE ON THE MESENCHYMAL STEM/STROMAL CELL ISOLATION YIELD

Natalia Siedlecka[P][1], Paulina Ośka[1], Arkadiusz Jundziłł[1], Tomasz Drewa[1], Marta Pokrywczyńska[1]

[1] Department of Regenerative Medicine Nicolaus Copernicus University in Torun Ludwik Rydygier Medical College in Bydgoszcz

Corresponding author's email: siedleckanatalia92@gmail.com

Adipose tissue is a highly specialized tissue which plays a number of functions in the organism. This tissue is also a perfect source of Adipose Derived Mesenchymal Stem/Stromal Cells (AD-MSCs). Main advantages of adipose tissue as a source of stem cells are high accessibility and abundance. AD-MSCs may be found either in subcutaneous or visceral adipose tissue. The aim of the study was a comparison of different anatomical adipose tissue harvesting sites on the mesenchymal stem/stromal isolation yield.

The adipose tissue was obtained from 14 male domestic pigs from three anatomically different harvesting sites: the subcutaneous layer of the neck, the subcutaneous inguinal layer and the preperitoneal layer. The cells were isolated through digesting the adipose tissue with collagenase P. The number and viability of isolated cells were evaluated. The success rate of establishing a primary culture of AD-MSCs, immunophenotyping and proliferation kinetics were assessed.

The overage numbers of isolated cells from the neck subcutaneous adipose tissue, inguinal subcutaneous adipose tissue, visceral adipose tissue were 579,179 (± 156,820), 2,232,339 (± 799,050) and 313,787 (± 179,968) cells respectively. All the AD-MSCs populations showed high expression of CD29, CD31, CD90 markers; and very low expression of CD11b, CD31 and CD45 markers.

The subcutaneous inguinal adipose tissue seems to be a better source of mesenchymal stem cells in a porcine model compared to other examined sources: the neck subcutaneous adipose tissue and visceral adipose tissue.

Keywords

AD-MSCs; adipose tissue

Abstract **#1938**

THERMOGELLING BIOMIMETIC HYDROGEL FOR 3D NEURONAL NETWORKS

Donatella Di Lisa[1,2], Elena Dellacasa[1,2], Lorenzo Muzzi$_P$[1], Alberto Lagazzo[3], Monica Frega[4], Sergio Martinoia[1], Laura Pastorino[1,2]

[1] University of Genova, Dept. of Informatics, Bioengineering, Robotics and System Engineering, Via Opera Pia 13, 16145 Genova, Italy

[2] Bio3DmatriX s.r.l., Via di San Pietro della Porta 23, 16123, Genova, Italy

[3] University of Genova, Dept. of Civil, Chemical and Environmental, Via Opera Pia 15, 16145 Genova, Italy

[4] University of Twente, Dept. of Clinical neurophysiology, University of Twente, Drienerlolaan 5, 7522 NB Enschede,

Corresponding author's email: lorenzo.muzzi.93@gmail.com

Engineered natural biomaterials able to perform the sol-gel transition under specific external stimuli, also in the presence of cells, have gained great attention in the field of tissue engineering and regenerative medicine [1]. Despite of this, the encapsulation of neurons for the development of 3D neuronal networks, is still limited. In order to study neuronal (dys)functions and particularly neuronal connectivity, in vitro human brain models need to include not only a chemically and physically relevant microenvironment, but also structural network complexity [2]. To mimic the brain ECM environment, a porous and soft structure is preferred in the design of an artificial neural network. The goal of this study was to demonstrate the versatility of thermosensitive chitosan-based scaffold as an artificial matrix for 3D neuronal networks for in vitro studies and as an injectable-hydrogel for the in-vivo applications or innovative ink for 3D bioprinter. Chitosan is a widely used biomaterial, well known for its biocompatibility, biodegradability, muco-adhesiveness as well as its antibacterial activity [3]. It is already demonstrated that chitosan is able to sustain the development of 3D scaffold for neuronal networks [4]. In this work, chitosan thermogels were fabricated and characterized by different techniques. Primary rat neurons and human-induced neurons were mixed within chitosan thermogelling solution prior the scaffold gelation. The 3D neuronal cultures were morphological characterized by immunofluorescence techniques and a preliminary electrophysiological characterization of spontaneous activity by Micro-Electrode Arrays, was carried out

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Abstract **#1939**

OPTIMISATION AND UP-SCALING PRODUCTION OF HUMAN WHARTON'S JELLY MESENCHYMAL STEM CELLS IN SPINNER FLASK UNDER XENO-FREE ENVIRONMENT

Benson Koh[P][1,2], Muhammad Dain Yazid[1], Nadiah Sulaiman[1], Mh Busra Fauzi[1], Jia Xian Law[1], Min Hwei Ng[1]

[1] *Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre*

[2] *Ming Medical Sdn Bhd*

Corresponding author's email: benson9584@gmail.com

Mesenchymal stem cells (MSCs) have been used in cell transplantation and tissue engineering for their self-renewal and multipotency capabilities. The conventional stem cell expansion normally performed on a two-dimension surfaces such as culture flasks which take up large number of spaces. In this study, different supplements were used to identify a scalable xeno-free culture environment for MSCs proliferation. The cell yield, cell surface markers, cell plasticity, and immunomodulation have been compared against those cultured in standard fetal bovine serum supplemented medium. The MSCs cultured in human platelet lysate supplemented medium exhibit highest cell yield with comparable cell characteristic aforementioned to the control. In spinner flask culture, suitable culture parameter has been optimised to maximise the yield of MSCs. MSCs were cultured on three different microcarriers (Cytodex 1 (C1), Cytodex 3 (C3), Corning collagen coated microcarrier (CCM)) for cell expansion. At initial seeding process, visible aggregates were observed in C3 and CCM, whereas MSCs cultured on C1 formed minimal aggregation throughout the culture period. The results of current study demonstrate the feasibility of scale-up production of MSCs in spinner flask under xeno-free environment. It also highlights the potential of bioreactor in future MSCs up-scaling production for tissue engineering application.

Keywords

Up-scaling Production; Microcarriers; Xeno-free

Abstract **#1940**

IN VIVO EVALUATION OF A BILAYER SCAFFOLD FROM PLGA/FIBRIN AND FIBRIN HYDROGEL USED AS A SKIN SUBSTITUTE

Patricia Pranke[1,2], Juliana Girón$_P$[1,2], Luiza Oliveira[2], Bruno Alcantara[2], Fabíola Meyer[3], Natasha Maurmann[2,1]

[1] *Post Graduate Program in Physiology, Universidade Federal do Rio Grande do Sul*

[2] *Hematology & Stem Cell Laboratory, Faculty of Pharmacy, Universidade Federal do Rio Grande do Sul*

[3] *Biochemistry Department, Universidade Federal do Rio Grande do Sul*

Corresponding author's email: jgironb@unal.edu.co

Because the incidence of skin wounds requiring clinical treatment represents a public health problem worldwide, the present work aims to develop a bilayer scaffold of PLGA/fibrin electrospun membrane and fibrin hydrogel layer to be tested in vivo as skin substitutes. Primary cultures of fibroblasts and keratinocytes were isolated from isogenic Wistar Kyoto rats (WKY). Fibroblasts were cultivated in the fibrin hydrogel and keratinocytes on the electrospun membrane to generate a skin substitute using an air/liquid system. The scaffolds were tested in a full-thickness wound model in WKY rats of 3 months. Three groups were analyzed macroscopically: 1 (bilayer scaffold without cells), 2 (heterotypic skin substitutes), 3 (negative control). Results showed scab formation at day 14 in all animals from groups 1, 2, and 3. No signs of wound infection were presented. Regarding hair growth, 71% of the lesions in group 2 presented hair growth at day 14, while no hair growth was seen in groups 1 and 3. Additionally, distant suture points were seen in groups 1 and 2, which suggest skin regeneration and little wound contraction on both days 14 and 21. On day 21 all the wounds were re-epithelialized and presented hair. It could be concluded that the bilayer scaffold is thus a promising matrix for use as a skin substitute. However, it will be necessary to complete the sample size for each group and realize histological and immunoenzymatic assays to confirm the regeneration of the tissue. Financial support: CAPES, MCTIC, FINEP, CNPq, and Stem Cell Research Institute.

Keywords

Scaffold; fibrin; PLGA

Abstract **#1941**

COMBINING BIO-ENGINEERED HUMAN SKIN WITH BIO-PRINTED CARTILAGE FOR EAR RECONSTRUCTION

Dominika Zielinska$_P$[1,2], Philipp Fisch[3], Marcy Zenobi-Wong[3], Thomas Biedermann[1,2], Agnes Klar[1,2]

[1] *University Children's Hosptal Zurich, Tissue Biology Research Unit, Zurich, Switzerland*

[2] *Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland*

[3] *Department of Health Science and Technology, ETH Zurich, Zurich, Switzerland*

Corresponding author's email: dominikazielinska94@gmail.com

Purpose: Microtia is a congenital disorder manifested by an external ear malformation or a lack of the entire external ear auricle (anotia) [1]. The aesthetic reconstruction of the auricle remains a significant clinical problem. Children suffering from microtia often require repeated reconstructive follow-up operations [2, 3]. Therefore, we developed a novel tissue-engineered based reconstruction approach for microtia treatment.

Methods: Human auricular chondrocytes were bioprinted within a hyaluronan transglutaminase (HATG) bioink and combined with a bio-engineered human pigmented and prevascularized dermo-epidermal skin substitute [4, 5, 6]. Human fibroblasts and endothelial cells were used to develop a collagen type I-based dermal component with keratinocytes and melanocytes seeded on the surface. The cartilage constructs were transplanted onto immune-incompetent rats with pre-vascularized, pigmented skin substitutes on top. After short (1 week) and long-term (5 weeks) studies, the grafts were excised and used for histological and immunofluorescence analysis.

Results: Short-term in vivo tests have confirmed the anastomoses of the engineered blood capillaries with the recipient's vasculature enabling a rapid perfusion. Long-term in vivo tests have demonstrated that bioprinted material creates mechanically stable cartilage. Histological staining of the cartilage implant demonstrated extracellular matrix with glycosaminoglycans and collagen type II. In addition, bio-engineered skin displayed a stratified epidermis containing keratinocytes and melanocytes distributed throughout the basal layer that efficiently restored the donor native skin color.

Conclusions: Bioprinted auricular cartilage combined with a bio-engineered human pigmented and prevascularized dermo-epidermal skin substitute represents a novel and promising treatment option for children born with microtia.

Keywords

Bio-Engineered Skin; Bio-Printed Cartilage; Microtia

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Abstract **#1942**

CONTROLLED RELEASE OF ROD-DERIVED CONE VIABILITY FACTOR FROM AN INJECTABLE HYDROGEL DRUG DELIVERY SYSTEM

Carter Teal[P1], Margaret Ho[1], Lia Huo[2], Hidekiyo Harada[3], Thierry Léveillard[4], Philippe Monnier[3,5], Molly Shoichet[1,2,6]

[1] *Institute of Biomedical Engineering, University of Toronto, Canada*

[2] *Institute of Medical Science, University of Toronto, Canada*

[3] *Vision Division, Krembil Research Institute, Canada*

[4] *Department of Genetics, Institut de la Vision, Inserm, Université Pierre et Marie Curie Paris 6, France*

[5] *Department of Physiology, Faculty of Medicine, University of Toronto, Canada*

[6] *Department of Chemical Engineering and Applied Chemistry, University of Toronto, Canada*

Corresponding author's email: carter.teal@mail.utoronto.ca

Introduction

Retinitis pigmentosa (RP), the leading cause of hereditary blindness, is characterized by the degeneration of rod photoreceptors and subsequent loss of cone photoreceptors. Rod-derived cone viability factor (RdCVF) is a protein paracrine factor which maintains the structure and function of cone photoreceptors (1). RP patients may benefit from exogenous application of RdCVF; however, effective administration of protein therapeutics to the retina remains challenging. Topical applications are limited by bioavailability and bolus injection by risk of infection. To address these challenges, we developed an injectable hydrogel composed of hyaluronan and methylcellulose (HAMC) (2,3) modified with peptide binding partners of the Src homology 3 (SH3) domain and expressed a fusion protein of SH3-RdCVF, enabling its controlled release.

Methods

SH3-RdCVF was dispersed into a HAMC hydrogel modified with SH3 binding peptides immobilized by Michael addition between the maleimide-functionalized peptides and thiolated methylcellulose polymer chains. Sustained release was investigated in vitro for 7 days, quantified using a custom ELISA, and its bioactivity tested with chick retinal dissociates (E6).

Results

The sustained release of SH3-RdCVF was achieved by modulating the affinity between SH3 and its binding peptides for at least 7 days. Moreover, released SH3-RdCVF was bioactive as demonstrated with a significant increase in the viability of cone-photoreceptor cells in vitro.

Conclusions

For the first time, RdCVF was incorporated into a hydrogel from which its controlled, bioactive release was demonstrated, thereby laying the foundation for its use in vivo. Our affinity-based system constitutes a versatile delivery platform for the treatment of retinal degenerative diseases.

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Abstract **#1943**

MESENCHYMAL STEM CELL THERAPY IMPROVES CLINICAL OUTCOMES IN THE OSTEOARTHRITIS OF THE HUMAN KNEE

Christopher C.H. Mak[P][1], Nikhil Agarwal[2], Christine Bojanic[3], Kendrick To[3], Wasim Khan[3]

[1] *School of Clinical Medicine, University of Cambridge*

[2] *University of Aberdeen College of Life Sciences and Medicine*

[3] *Division of Trauma & Orthopaedic Surgery, Addenbrooke's Hospital, University of Cambridge*

Corresponding author's email: makchchris@gmail.com

Osteoarthritis (OA) is a leading cause of disability around the world. With an aging population and increased incidence of obesity the burden of OA is set to rise, paving way for intra-articular mesenchymal stem cell (MSC) therapy.A PRISMA systematic review and meta-analysis was conducted employing four databases (MEDLINE, EMBASE, Cochrane, Web of Science) to identify all clinical studies which utilised adipose tissue derived MSCs or SVF (stromal vascular fraction) for the treatment of knee OA. In particular, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was analysed for quantifiable outcomes.Eighteen studies were found which met the inclusion criteria. Eleven studies investigated the effects of adipose tissue derived MSC treatments on knee osteoarthritis, while eight studies examined SVF. All studies found that both therapies are effective in reducing pain and improving knee function in patients with knee OA. When stratifying studies by the change in WOMAC across time, the studies reported a 17.3%[CI 2.4-32.1%] decrease at ≤1 month, 39.6%[CI 29.0-50.2%] decrease at 2 months, 36.6% [CI 21.4-51.7%] at 3 months, 41.3% [CI 29.9-52.6] at 6 months, 35.9%[CI 11.8-60.0%] at 12 months, 65.7%[CI 51.3-80.1%] at 18 months, and 62.1%[51.5-72.7%] at 24 months. These results suggest MSC treatments on knee OA provide long-term pain relief and improved joint function, with a dose-dependent effect.The studies in this systematic review have established the safety and efficacy of both adipose tissue derived MSC and SVF therapy for knee OA in humans.

Keywords

Stem Cell Therapy; Clinical; Osteoarthritis

Abstract **#1944**

ELECTROSPRAYING TECHNIQUE FOR THE DEVELOPMENT OF NANOCAPSULES CONTAINING ANTICOAGULANT

Patricia Pranke[1,2,3], Bruna Leal[1,2], Daikelly Braghirolli[2]

[1] *Post Graduate Program in Physiology, Universidade Federal do Rio Grande do Sul*

[2] *Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Universidade Federal do Rio Grande do Sul*

[3] *Stem Cell Research Institute*

Corresponding author's email: patriciapranke2@gmail.com

Electrospraying (ES) technique is widely used in the development of nanocapsules (NC) for use in tissue engineering (TE), allowing for encapsulation of biomolecules. In this work, NC containing the anticoagulant heparin (Hep) were developed by ES, with the aim of their future association with vascular TE grafts. NC were developed using an emulsion of poly (lactic-co-glycolic acid) (PLGA) by the ES technique, with 0 (Control/NC) or 1000 IU of Hep (Hep/NC). The size, polidispersion (PdI) and zeta potential of the NC were analyzed by ZetaSizer, and their morphology, by scanning electron microscopy. The efficiency of encapsulation (EE) and release of Hep were determined by the turbidimetric method. The functionality of Hep/NC was analyzed by activated partial thromboplastin time (aPTT). The Control/NC and Hep/NC showed round and homogeneous morphology. The control/NC showed of mean diameter of 178.8±3.7 nm, 0.326±0.04 of PdI and -23.4±1.5 mV of zeta potential. The Hep/NC showed 262.6±14.6 nm of diameter, 0.291±0.01 of PdI and -28±2.5 mV of zeta potential. The EE was 98.9%. In the release test, most of the Hep was released in the first hour. However, after this, the release was gradual and controlled. In the functionality test, the blood incubated with Hep/NC did not coagulate. The NC were successfully produced by the ES technique, with a high EE rate. However, this process did not alter the activity of the Hep. Electrosprayed NCs can be easily associated with TE biomaterials, increasing their success in their application as vascular grafts.

Keywords

Electrospraying; anticoagulant; nanocapsules

Abstract **#1945**

ACELLULAR MATRIX GRAFTS TO AUGMENT THE PERI-URETHRAL TISSUE BED FOR HYPOSPADIAS REPAIR

Debora Morgante[P][1,2,3], Anna Radford[2,3], Syed Khawar Abbas[4], Eileen Ingham[5], Ramnath Subramaniam[2], Jennifer Southgate[1]

[1] *Jack Birch Unit, Department of Biology, University of York, York, UK*

[2] *Paediatric Urology, Leeds Teaching Hospitals NHS Trust, Leeds, UK*

[3] *Paediatric Surgery, Hull University Teaching Hospitals NHS Trust, Kingston upon Hull, UK*

[4] *Central Biomedical Services, University of Leeds, Leeds, UK*

[5] *Institute of Medical and Biological Engineering, University of Leeds, Leeds, UK*

Corresponding author's email: debora.morgante.md@gmail.com

Introduction

Hypospadias is a common birth defect requiring surgical repair and associated with long-term complications. Cross-linked or non-cross-linked decellularised tissue matrices were hypothesised to provide natural substrates to augment the tissue bed in surgical situations where the native tissue bed is insufficient to support an enduring repair.

Methods

Single patches of acellular, non-cross-linked biomaterial developed from full-thickness porcine urinary bladders (Porcine Acellular Bladder Matrix; PABM) or commercial cross-linked acellular matrix derived from porcine dermis (Permacol™) were implanted in a peri-urethral position in male pigs. After three months, macroscopic and microscopic evaluations including a novel objective quantitative analysis of immunohistochemically-labelled tissue sections were performed to assess tissue integration outcomes.

Results

All pigs survived the post-operative period with no complications. Macroscopic evaluation revealed no evidence of residual PABM, but Permacol™ remained evident. Microscopically, there was no sign of any adverse or inflammatory tissue reaction. Permacol™ grafts showed evidence of partial encapsulation and very sparse cell infiltration. By contrast, PABM implants showed uniform cellularisation, including neovascularisation, throughout the implanted biomaterial. Total cell counts, as well as the densities of infiltrating cells, resulted significantly higher in PABM than in Permacol implanted grafts.

Discussion

The strong, compliant structural and integrative properties of PABM support its use in surgical situations where there is an insufficient tissue bed to withstand repair. In particular, we propose an application in primary complex hypospadias repair, where rapid integration as a supportive layer is predicted to reduce complications and the need for revision surgery.

Keywords

acellular matrices; hypospadias; tissue regeneration

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Abstract **#1946**

A PHYSIOLOGICAL 3D DYNAMIC MECHANICAL LOADING PLATFORM FOR MENISCUS TISSUE ENGINEERING

Jing SUN$_P$[1,2], Yau Tsz CHAN[1,2], Ki Wai Kevin HO[3], Sung Chi Rocky TUAN[1,2], Yangzi JIANG[1,2]

[1] *Institute for Tissue Engineering and Regenerative Medicine, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong*

[2] *School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong*

[3] *Department of Orthopaedics & Traumatology, Prince of Wales Hospital, Faculty of Medicine, The Chinese University of Hong Kong*

Corresponding author's email: sunjing9302@link.cuhk.edu.hk

Meniscus tears are frequently encountered in clinical. As a load-bearing tissue, the dynamics of meniscus repair in vivo are governed by both biological and biomechanical cues. Biocompatible hydrogel and tissue specific stem/progenitor cells are the most physiological mimetic elements that have been used in meniscus tissue engineering1. In this study, we have established a homemade GELMA hydrogel-based bioreactor, which can provide repetitive mechanical loading to 3-dimensional (3D) cultured human meniscus progenitor cells (hMeSPCs)2, mimicking the mechanical microenvironment of meniscus tissue in vitro. hMeSPCs (n=9, from hospital with ethical approval) were isolated by multiple colony selection, and expanded in vitro. The cells showed 12.5% of CFU, and were characterized by flowcytometry for stem cell surface markers (CD90/73/105/44/45/34). Controlled tensile strain (10% hydrogel deformation, 0.5 Hz, 1 h/day, loading group)3 was applied to the Gelatin Methacryloyl (GelMA) hydrogel-encapsulated cells through the bioreactor, which enabled the 3D-cell culture of hMeSPCs for more than 15 days with 94% of the cell survival rate, examined by PI staining, flowcytometry. The loading enhanced the differentiation of encapsulated hMeSPCs, and increased the extracellular matrix (ECM) deposition, compared with the static group (n=9, pooled cells into 3 batches, $p<0.05$). Furthermore, we evaluated the gene expression levels of 3D static and loading groups by RNA-seq (n=6, 2 batches), and 198 genes were identified that are regulated by the dynamic mechanical loading. Together, we here report a functional physiological 3D dynamic mechanical loading platform for meniscus tissue engineering. (Supported by National Key R&D Program of China, 2019YFA0111900).

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Abstract **#1947**

MODULATING HYDROPHILIC CELLULOSE ACETATE AND POLYETHERSULFONE TO HYDROPHOBIC: EFFECTIVE IMMUNOISOLATION DEVICES DEVICE FOR ALLOGENEIC ISLET CELL TRANSPLANTATION.

Gokula Nathan Nathan Kasinathan$_P$[1], Pavan Kumar P[2], Sasikala Mitnala[2], Aakanksha Ruhela[3], Chandra Shekhar Sharma[3], Subha Narayan Rath[1]

[1] Regenerative Medicine and Stem Cell Laboratory (RMS), Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Kandi-502285, Telangana, India.

[2] Asian Healthcare Foundation, Gachibowli, Hyderabad - 500032, Telangana, India

[3] 3Creative & Advanced Research Based On Nanomaterials (CARBON) Laboratory, Department of Chemical Engineering, Indian Institute of Technology Hyderabad, Kandi-502285, Telangana, India.

Corresponding author's email: bm17resch11005@iith.ac.in

Allogeneic Islet transplantation holds appreciable recovery towards the normoglycaemia in T1D patients; but the greatest challenge is to have an ideal encapsulation device. An effective device, firstly never eliciting a foreign body response, ensures the encapsulated cells viable, transducing the signals; yet protecting from the host immune responses [1]. To overcome the challenges, we tuned the physical features of the Cellulose acetate (CA) and Polyethersulfone (PES) through electrospinning technique, assessed in vitro and in vivo. Fabricated nano-porous membrane devices expressed the ability of selective immune-isolation property, insulin secretion to glucose levels and ensured the encapsulated cells functionality. Electrospinning resulted a modified surface wettability converting the hydrophilic CA (136°) and PES (126°) to hydrophobic [2]. Enhanced hydrophobicity helped in maintenance of the 3D morphology of the L929 cells and Min6 Spheroids. SEM evidenced the nano-topographical features effectively stopped the monocyte to macrophage conversion even after 96 hours; complimenting the mRNA levels. All nano-fibrous membranes ceased the migration of Thp-1 Monocytes in a trans-well migration assay mimicking a xenogeneic model. Further GSIR studies revealed the efficient insulin release upon glucose stimulation in vitro with Min6 and rat islets. In vivo implantation of CA and PES devices in allogeneic rat models encapsulated with rat islets found to be insulin independent even after 4 weeks, successful engraftment with visible vasculatures around the devices makes them promising for encapsulation technology. Thus exploiting topography and physical features can envisage us to have an ideal encapsulation device for immune-isolation therapies.

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Abstract **#1948**

REPAIR OF INTRAUTERINE ADHESIONS BY USING ENZYMATICALLY CROSSLINKED GELATIN HYDROGEL LOADED WITH HUMAN MENSTRUAL BLOOD-DERIVED STROMAL CELLS

Fengyu Wang[P][1]

[1] *School of Medicine, South China University of Technology, Guangzhou, China*

Corresponding author's email: mswangfengyu@mail.scut.edu.cn

Approximately 25-30% of infertility is caused by intrauterine adhesions (IUA; also known as Asherman syndrome) [1]. A traditional treatment for IUA is resection of adhesion by hysteroscopy. However, it fails to prevent IUA recurrence and endometrial scarring formation. In present study, we aim to investigate the therapeutic effects of transplanting of human menstrual blood-derived stromal cells (MenSC) combined with enzymatically crosslinked gelatin hydrogel (enGel) in rat IUA models and the possible mechanisms in the treatment of endometrium regeneration. Particularly, we record endometrial morphology and pathology, tissue proliferation, inflammation, pregnancy outcomes etc. Our results suggest that enGel could partially repair IUA caused by mechanical injury, more interestingly, MenSC/enGel complex transplantation has notable dual repair effects: a reliable antiadhesion property and the remarkable promotion of endometrial regeneration.

Keywords

menstrual blood-derived stromal cells; intrauterine adhesions; enzymatical crosslinking

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Abstract **#1949**

NANOROOMBA: PARTICLE SURFACES AS INSTRUCTIVE BIOINTERFACES

Aaron Lee$_P$[1], Dedy Septiadi[1], Patricia Taladriz-Blanco[1], Barbara Rothen-Rutishauser[1], Alke Petri-Fink[1,2]

[1] *Adolphe Merkle Institute, University of Fribourg*

[2] *Chemistry Department, University of Fribourg*

Corresponding author's email: aaron.lee@unifr.ch

The physical environment sensed by cells is central to defining their behaviour. To understand how local environmental features may influence cellular responses such as attachment, adhesion, proliferation, migration and differentiation, we have implemented a method by which surface characteristics are parameterised through particle synthesis and controlled assembly. We show that such particle-decorated surfaces are not only cell-relevant, but can act as instructive interfaces. Negatively charged silica particles adsorbed to a poly(L-lysine) treated surface are removable by A549 lung adenocarcinoma cells only when the particle size is greater than 480 nm, while human monocyte-derived macrophages are able to remove particles as small as 180 nm. Additionally, stiffness-dependent effects are manifest with preferential interactions supported by more rigid particles when presented with differentially crosslinked poly(N-isopropylacrylamide) particles. Confocal microscopy has indicated that in response to these particle surfaces, alterations to adhesion complex formation are apparent. Localisation of paxillin and vinculin to the particles highlights how topographical cues are sensed by the cells and guide their response. To highlight how such particle surfaces might guide behaviour, we use surface particle removal as a novel method for the introduction of functional molecules into adherent cells. Using these insights, we show how different cell instructive interfaces based on controlling particle properties and location can be developed and deployed.

Abstract **#1950**

DEVELOPMENT OF BIOACTIVE HYDROGELS FOR NERVE TISSUE REGENERATION

ozgul gokP[1], Ali Murad Özmen[2]
[1] *Assist. Prof. at Acibadem University*
[2] *Acibadem University*
Corresponding author's email: ozgul.gok@acibadem.edu.tr

INTRODUCTION: Hydrogels are promising polymeric scaffolds as they provide biocompatible and biodegradable network structures for the growth of cells together with necessary growth factors and desired cell adhesion peptides.1 In this study, different peptides were immobilized into biodegradable PEG-based hydrogels and in vitro studies were conducted to demonstrate their biological efficiency towards especially for nerve cell growth and proliferation.2

METHOD: PEG-based hydrogels were synthesized under UV light (365nm) using thiol-yne click chemistry. Hydrogels were added with PLA (5-10-20% by weight) and hyaluronic acid (10-20-30% by weight) for adjusting the stiffness of resultant scaffold to be suitable for neural tissue. Also two cell adhesion peptides were introduced into scaffold to enhance nerve cell growth and proliferation.

RESULTS:

Figure 1. SEM image of PEG-based hydrogel with 10%PLA and %0.1RGD peptide.

DISCUSSION AND CONCLUSION:
The obtained hydrogels were characterized by FT-IR for their functional groups and mechanical behavior was analyzed by reometer. SEM results indicated that all hydrogels have porous structure and they were shown to be non-toxic over L929 fibroblast cell line (>85% cell viability).

ACKNOWLEDGEMENTS:
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Keywords
Bioactive hydrogels; peptide conjugation; artificial scaffold

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Abstract **#1951**

CRANIOFACIAL BONE DEFECT REPAIR USING POLYMER SCAFFOLDS AND CELL DERIVED MATRIX

Witchayut Sasimonthon$_P$[1], Amy Leigh Harding[2], Samand Pashneh-Tala[1], Frederik Claeyssens[1], Helen Colley[2], Gwendolen Clair Reilly[1]

[1] *Department of Materials Science and Engineering, University of Sheffield*

[2] *School of Clinical Dentistry, University of Sheffield*

Corresponding author's email: wsasimonthon1@sheffield.ac.uk

Autografts, the gold standard for craniofacial repair but have limited source tissue and harvested site morbidity. Tissue-engineering has the potential to facilitate craniofacial bone regeneration but advanced materials that can address these limitations are warranted. Poly(glycerolsebacate) (PGS) has been investigated for soft tissue regeneration, but not been widely studied for bone. Its mechanical properties can easily be tailored whilst being biocompatible. Cell-derived extracellular matrix (ECM) has been shown to enhance osteoprogenitor cell proliferation, differentiation and mineralisation(1). However, it has limited mechanical properties and cannot be shaped to fill complex bone defects. Therefore, we aim to create 3D bone-like tissue for craniofacial repair using a combination of PGS and cell-derived ECM. PGS were synthesised using a porogen-leaching technique with different ratios (1:2.5- 1:4.5) of prepolymer to salt (w/w), porosity assessed using pycnometry and scanning electron microscopy (SEM). Y201 (mesenchymal stem cell (MSC) cell line) attachment and matrix deposition onto the scaffolds were investigated by resazurin reduction assay and Western blot over 21 days. It showed that the ratios used produced a range of pore densities, with a higher salt content producing higher porosity. While pore sizes are between 50-400 µm, suitable for cell attachment and growth(2). The scaffolds supported cell attachment (up to 65%), with slightly higher attachment on lower porosity scaffolds. Furthermore, western blotting confirmed that the scaffolds supported deposition of key bone proteins including collagen, fibronectin, bone-sialoprotein 2, and osteopontin. In summary, the scaffolds support MSC growth and bone matrix deposition and have potential applications in craniofacial bone regeneration.

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Abstract **#1952**

EFFECT OF HYPOXIA MIMICKING RESPONSES ON VASCULAR REGENERATION

Muhammad Shafiq[P][1], Muhammad Rafique[1], Onaza Ali[2], Tingting Wei[1], Kai Wang[1], Deling Kong[3]

[1] *Institute of Biochemistry & Molecular Biology, College of Life Sciences, Nankai University, Tianjin, China*

[2] *State Key Laboratory of Separation Membranes and Membrane Processes, School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin, China*

[3] *Institute of Biochemistry & Molecular Biology, Nankai university, Tianjin, China*

Corresponding author's email: muhammad.shafiq917@yahoo.com

Abstract:

Cellular transition to hypoxia following tissue injury, has been shown to improve angiogenesis and regeneration in multiple tissues. To take advantage of this, many hypoxia-mimicking scaffolds have been prepared, yet the oxygen access state of implanted artificial small-diameter vascular grafts (SDVGs) has not been investigated. Therefore, the oxygen access state of electrospun PCL grafts implanted into rat abdominal arteries was assessed. The regions proximal to the lumen and abluminal surfaces of the graft walls were normoxic and only the interior of the graft walls was hypoxic. In light of this differential oxygen access state of the implanted grafts and the critical role of vascular regeneration on SDVG implantation success, we investigated whether modification of SDVGs with HIF-1α stabilizer dimethyloxalylglycine (DMOG) could achieve hypoxia-mimicking responses resulting in improving vascular regeneration throughout the entirety of the graft wall. Therefore, DMOG-loaded PCL grafts were fabricated by electrospinning, to support the sustained release of DMOG over two weeks. In vitro experiments indicated that DMOG-loaded PCL mats had significant biological advantages, including: promotion of human umbilical vein endothelial cells (HUVECs) proliferation, migration and production of pro-angiogenic factors; and the stimulation of M2 macrophage polarization, which in-turn promoted macrophage regulation of HUVECs migration and smooth muscle cells (SMCs) contractile phenotype. These beneficial effects were downstream of HIF-1α stabilization in HUVECs and macrophages in normoxic conditions. Our results indicated that DMOG-loaded PCL grafts improved endothelialization, contractile SMCs regeneration, vascularization and modulated the inflammatory reaction of grafts in abdominal artery replacement models, thus promoting vascular regeneration.

Abstract **#1954**

INVESTIGATION OF THE TRANSFECTION CAPABILITIES AND CELLULAR UPTAKE OF PLASMID DNA AND MODIFIED MRNA FOR TISSUE ENGINEERING APPLICATIONS

Claudia Del Toro Runzer[P][1], Christian Plank[2], Elizabeth Rosado Balmayor[3], Martijn van Griensven[1]

[1] *CBITE Department, MERLN Institute for Technology-Inspired Regenerative Medicine*

[2] *Ethris GmbH*

[3] *IBE Department, MERLN Institute for Technology-Inspired Regenerative Medicine*

Corresponding author's email: c.deltororunzer@maastrichtuniversity.nl

Nucleic acids have gained much popularity due to their clinical potential for gene therapy and regenerative medicine. Plasmid DNA (pDNA) was the first nucleic acid to be pursued as a therapeutic molecule and remains the most used for cellular modification. Later on, several RNAs came into play as they offer several advantages: complete degradation, avoid transport across the nuclear membrane, and do not hold risks associated to genome integration. It is generally accepted that both mRNA and pDNA cellular internalization occurs via endosomal uptake and cytosolic release. pDNA needs nuclear entry. Nevertheless, their mechanistic process of cellular uptake remains poorly understood.

In this study, we investigated the transfection efficiency and protein expression kinetics of two nucleic acids, i.e. pDNA and chemically modified mRNA (cmRNA). The reporters Metridia Luciferase (MetLuc) and enhanced green fluorescent protein (eGFP) were used. pDNA and cmRNA were delivered using two lipid vectors in HEK293 and hMSCs. Protein expression and biocompatibility were evaluated spectrophotometrically at up to 3 days post-transfection. Mechanisms of cellular uptake were studied by endocytosis inhibition whereas cellular internalization was investigated by Correlative Light and Electron Microscopy. Results in HEK293 showed highest expression when pDNA lipoplexes (lipofectamine) was used. Interestingly, in primary cell, the best performance was observed with the vector 3DFect and cmRNA. Additionally, while mRNA-transfected cells showed an early peak of MetLuc activity (day 1), pDNA transfection resulted in a later onset of expression with peaks at day 2 and day 3 for HEK293 and hMSCs, respectively.

Funding: H2020 No. 874790 (cmRNAbone).

Abstract **#1955**

TOWARDS ELASTIC CARTILAGE IN BIOPRINTED AURICLES

Philipp Fisch[P][1], Killian Flégeau[1], Domiinika Zielinska[2], Agnes Klar[2], Sergio Finkielsztein[3], Thomas Linder[4], Thomas Biedermann[2], Marcy Zenobi-Wong[1]

[1] *Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland*

[2] *Tissue Biology Research Unit, University Children's Hosptal Zurich, Zurich, Switzerland*

[3] *Marine Polymer Technologies, Inc., MA, USA*

[4] *HNO Luzerner Kantonsspital, Luzern, Switzerland*

Corresponding author's email: philipp.fisch@hest.ethz.ch

Bioprinting of functional tissue poses a major challenge as the process cannot reproduce the intricate complexity of native tissue and post-printing tissue maturation is required. We recently reported on a novel mechanism for enzymatically crosslinking bioinks,[1] where the outstanding chondrogenic properties of the hydrogel hyaluronan transglutaminase[2] was translated to cartilage bioprinting. However, while bioprinted constructs reached compressive moduli of up to 400 kPa and were stable in vivo for 6 months, the tissue displayed a tendency to develop towards fibrocartilage.

To steer tissue maturation towards elastic cartilage and to preserve the chondrogenic capacity of human auricular chondrocytes, we altered the bioink's composition to include a low percentage of alginate and explored the addition of various growth factors such as FGF-2 and TGF-β3. Matured constructs showed an abundance in glycosaminoglycans, collagen II and elastin without the presence of collagen I, as observed in human auricular cartilage. The mechanical properties of these constructs further reached up to 1 MPa, approximately two thirds of the compressive modulus of native human auricular cartilage, after 9 weeks in culture.

By replicating elastic cartilage, these results demonstrate a significant step towards the treatment of microtia patients. However, challenges remain in upscaling to larger constructs as nutrient availability limits tissue maturation. To overcome these limitations, we explore methods to incorporate nutrient channels which improve tissue maturation throughout the construct. Lastly, to reduce skin tension on the bioprinted auricle, we combine bioprinted cartilage with tissue engineered skin[3,4].

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Abstract **#1956**

DOSE-CONTROLLED LOW-INTENSITY PULSED ULTRASOUND TO MODULATE INFLAMMATORY RESPONSE

Francesco Iacoponi[1,2], Francesco Fontana[1,2], Enrico Catalano[1,2], Cristina Manferdini[3], Diego Trucco[1,2,3], Andrea Cafarelli[1,2], Gina Lisignoli[3], Leonardo Ricotti[1,2]

[1] The BioRobotics Institute, Scuola Superiore Sant'Anna, 56127 Pisa, Italy

[2] Department of Excellence in Robotics & AI, Scuola Superiore Sant'Anna, 56127 Pisa, Italy

[3] IRCCS Istituto Ortopedico Rizzoli Laboratorio di Immunoreumatologia e Rigenerazione tissutale, , Via di Barbiano 1/10, 40136 Bologna, Italy

Corresponding author's email: Francesco.Iacoponi@santannapisa.it

Low-Intensity Pulsed Ultrasound (LIPUS) is an intriguing tool that allows providing cells and tissues with mechanical energy in a non-invasive way, inducing beneficial bioeffects [1]. LIPUS demonstrated high potential in the regulation of inflammatory processes [2]. However, US set-ups for in vitro experiments are usually featured by considerable errors in the US dose delivered to cells [3]. Thus, the stimulation conditions in the state-of-the-art are not entirely comparable/reliable and the optimal ones are often unknown [4]. In this study we developed two controlled LIPUS systems able to guarantee precisely controlled US dose at the target cells modulating different parameters. We used to test this technology on a pro-monocytic, human cell line (U937) that was stimulated with 50 ng/mL of PMA for 48 h and induced to inflammatory conditions by treating it with 1 µg/mL LPS. Then, dose-controlled LIPUS stimuli were provided for 2 h, by varying US parameters. First, three different frequencies were screened (38 kHz, 1 MHz and 5 MHz). Then, the role of US intensity was evaluated, by varying it (25, 100, 250 and 450 mW/cm2). Finally, different duty cycles were applied (10%, 20%, 30%, 40%). Results showed that the optimal anti-inflammatory conditions, minimizing the release of inflammatory cytokines (IL-1β, IL-8 and TNF-α) with respect to the non-stimulated controls, were: 38 kHz, 250 mW/cm2 and a duty cycle of 20%. Future efforts will investigate the intracellular pathways activated by these stimuli. Furthermore, additional in vitro tests will be performed on other cell models of the immune system.

Keywords

Ultrasound; Immune cells; Anti-inflammatory effects

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Abstract **#1957**

RATIONAL DESIGN OF TEVGS: KINETIC MODELING OF PROTEIN ADSORPTION AND IN VITRO VALIDATION

Alejandra Riveros$_P$[1], Javier Cifuentes[1], Maria A. Rodríguez-Soto[1], Andres J. Garcia-Brand[1], Natalia A. Suárez V.[1], Monica Cuellar[1], Carolina Muñoz-Camargo[1], Juan C. Cruz[1], Juan C. Briceño[1,2]

[1] *Department of Biomedical Engineering, Universidad de los Andes, Bogotá, Colombia*

[2] *Fundación Cardioinfantil Instituto de Cardiología, Bogotá, Colombia*

Corresponding author's email: ra.riveros11@uniandes.edu.co

In Tissue-engineered vascular grafts (TEVGs) thrombogenicity is a common failure cause in small diameter applications. Herein, we aim to study the correlation between mechanical-hemodynamic-biochemical variables on protein adsorption processes responsible for thrombogenesis through computational simulations and in vitro models. Briefly, a two-way Fluid-Structure Interaction (two-way FSI) model in ANSYS Fluent-2019R3® and ANSYS Transient Structural® was linked to a kinetic model for albumin and fibrinogen adsorption on the TEVG surface implemented in COMSOL Multiphysics5.3®. Physiological pulsatile pressures were introduced as User Defined Functions (UDF). For the in vitro validation, we conducted fluorescent labeling of albumin and fibrinogen for further adsorption on a 1 mm diameter TEVG placed into a fluidic device under controlled physiological and pulsatile flow conditions where the proteins were circulated. The protein adsorption was allowed at different time spans. Surface protein concentration and distribution were evaluated by confocal microscopy. The two-way FSI model provided physiological-baseline velocity profiles and wall shear stresses. The kinetic model linked to the two-way FSI model showed time-spatial dependent protein concentration and saturation, thereby demonstrating the influence of wall properties and pulsatile flow. This was further confirmed by the collected confocal images. In both cases, fibrinogen saturation rates were faster than albumin. As fibrinogen is thrombogenic, our models provide a robust tool to conduct multiparametric studies of TEVGs and consequently improve their rational design.

Keywords

Multiphysics; Protein Adsorption; TEVGs

Abstract **#1958**

NATURAL FIBER-BASED BIOACTIVE FUNCTIONALIZATION OF POROUS BIO COMPOSITES: INSIGHTS FROM COCOA BEAN SHELL INCORPORATION INTO MINERALIZED POLY(LACTIC ACID)

Andres J. Garcia-Brand$_P$[1,2], Alejandro Maranon[3], Carolina Muñoz-Camargo[1], Juan C. Cruz[1], Alicia Porras[2]

[1] *Department of Biomedical Engineering, Universidad de los Andes, Bogota, Colombia*

[2] *Department of Chemical and Food Engineering, Universidad de los Andes, Bogota, Colombia*

[3] *Department of Mechanical Engineering, Universidad de los Andes, Bogota, Colombia*

Corresponding author's email: aj.garcia14@uniandes.edu.co

Combining biodegradable polymers with inorganic sulfate-minerals have shown encouraging results for bone conductivity. Nevertheless, several challenges are yet to be solved including modulation of oxidative stress and improvement of angiogenic potential and mechanical properties. An avenue to overcome such issues is to include bioactive-fillers to attenuate the signaling of reactive-oxygen-species of osteoclasts during bone resorption, while enhancing mechanical performance in terms of tensile and compressive strength. Natural-fibers main constituents have been successfully tested for biomaterial reinforcement and functionalization. In fact, natural additives not only act as reinforcement, but also improve surface-bioactivity as has been reported for Cocoa Bean Shells. A byproduct-waste of chocolate production (60.535tons/year) rich in polysaccharides and antioxidants. Hence, this work presents the formulation and the preliminary in-vitro evaluation of poly(lactic-acid)/Hydroxyapatite/Cocoa-Bean-Shells-based (PLA/HA/CBS) composites fabricated by solvent-casting/particle-leaching method. Structural analysis (FTIR) confirms absence of covalent-bonding between materials. Tensile modulus of composites shows a stiffer material when HA/CBS content is increased, approaching that of cortical bone. Antioxidant activity as assayed by DPPH demonstrates a marked radical-scavenging activity attributed to CBS presence. Metabolic activity measurements via MTT indicate low cytotoxicity (below 10%), however, platelet aggregation increased up to 40%. In summary, the developed composites hold a significant promise for biomaterial-engineering as their tensile properties are tunable, exhibit strong antioxidant activity and might induce angiogenic factors release for the potential treatment of bone defects. Further studies of the possible role of CBS as bioactive-filler are therefore encouraged including immune-response in-vivo. Moreover, this is an eco-friendly alternative to take advantage of chocolate-industry byproducts.

Keywords

Natural Fiber; Bioactive; Eco-friendly

Abstract **#1959**

MESENCHYMAL STROMAL CELL MODULATION OF THE SYNTHETIC BIOMATERIAL IMMUNE MICROENVIRONMENT

Karen Martin[P][1], Rebecca Schneider[1], Andrés García[1]

[1] *Georgia Institute of Technology*

Corresponding author's email: karen.martin@gatech.edu

The immune system plays a central role in orchestrating repair and regeneration of damaged tissue following disease or injury. Because of their immunomodulatory properties, mesenchymal stromal cells (MSCs) are an attractive cell source for use in diverse regenerative medicine applications. However, clinical translation is hampered by poor control over MSC survival, localization, and secretome upon transplantation in vivo. To overcome this, MSCs are often utilized in conjunction with biomaterial scaffolds (e.g. hydrogels), which are also known to dramatically alter the local immune landscape via host-biomaterial interactions. The combined impact of biomaterial and MSC delivery on the temporal recruitment and phenotypic shifts of immune cells within the local environment remains unclear.

We evaluated the effects of primary murine MSC co-delivery on the in vivo immune response to synthetic PEG-4MAL hydrogels in C57BL/6 immunocompetent mice by: (1) analyzing the local cytokine milieu via multiplex ELISA and (2) characterizing immune cell infiltration into the hydrogel over time via flow cytometry. Across all conditions, monocyte infiltration predominates at day 1, granulocytes peak around day 3, and macrophages are the predominate myeloid population by day 7. On day 3, two key chemokines, MCP-1, a potent monocyte chemoattractant, and MIP-2, a neutrophil chemoattractant, are present at significantly higher concentrations in IFNγ-stimulated MSC-laden hydrogels compared to acellular and unstimulated mMSC-laden hydrogels. A corresponding increase in the number of granulocytes and monocytes on day 1 and granulocytes and macrophages on day 3 was seen in the IFNγ-stimulated MSC-laden hydrogels compared to the other groups.

Abstract **#1960**

ENHANCED VASCULARIZATION GUIDED BY MICROPATTERNED POROUS FREE- STANDING MEMBRANE

Aakanksha Ruhela$_P$[1], Gokula Nathan Kasinathan[2], Subha Narayan Rath[2], Chandra Shekhar Sharma[1]

[1] *Creative & Advanced Research Based On Nanomaterials (CARBON) Laboratory, Department of Chemical Engineering, Indian Institute of Technology Hyderabad, Telangana, India*

[2] *Regenerative Medicine and Stem Cell Laboratory (RMS), Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Telangana, India*

Corresponding author's email: ch18resch01006@iith.ac.in

Vascularization of a scaffold or implant is one of the most fundamental challenges tissue engineering faces to successfully transplant engineered constructs. The blood vessels can provide the necessary nutrients and oxygen required for cell survival. Various strategies are being explored to enhance vascularization, including controlled release of growth factors. Modulating a scaffold's microarchitecture can also improve blood vessel formation because our native tissues are immensely organized at the microscale level. In the 1990s, Baxter Healthcare had already reported that biomaterial topography could influence blood vessel formation. In this work, we fabricate micro- patterned porous free-standing membranes by using photolithography and non- solvent-induced phase separation techniques. Random- pore membrane is taken as a control. SU-8 micropillars with 5µm and 40µm diameters are fabricated using photolithography. The fabricated micropillars are further used to produce patterned porous membranes via the phase separation method. Optical 3D profilometer and Scanning electron microscope (SEM) investigated the microarchitecture and surface topography of the membranes. The contact angle measurements reveal the hydrophilicity of the membranes, which will encourage cell attachment. The micropatterned membrane promotes endothelial cell proliferation in-vitro, a significant contributor to blood vessel formation. These findings validate the use of micro-patterned porous free-standing membranes to promote vascularization.

Keywords

Micro- patterning; Photolithography; Patterned porous membrane

Abstract **#1961**

PROFILING THE MICRORNA FINGERPRINT OF THE HUMAN FRACTURE HEMATOMA

Rald Groven[P][1,2], Bart van der Horst[1,2], Carlos Peniche Silva[1], Martijn Poeze[2], Taco Blokhuis[2], Martijn van Griensven[1]

[1] *Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands*

[2] *Division of Trauma Surgery, Department of Surgery, Maastricht University Medical Center+, Maastricht, The Netherlands*

Corresponding author's email: r.groven@maastrichtuniversity.nl

Immediately after a fracture occurs, a hematoma is formed. This hematoma plays an important role in fracture healing and, under healthy circumstances, aids in generating an environment in which a wide variety of cells orchestrate processes involved in fracture healing. MicroRNAs may influence these processes. The aim of this study was therefore to profile the expression of microRNAs in human fracture hematoma (fxH) samples.

fxH was harvested from 63 patients (mean age 51 ± 19; 32) during initial surgery. miRNAs were isolated, transcribed and pooled for qPCR array analysis. Qiagen fibrosis- and inflammation qPCR arrays were used based on an extensive literature study related to fracture healing and osteogenesis.

The array data revealed a tendency towards an anti-inflammatory miRNA signature in the fxH and showed a propensity for cytokine activity due to the downregulation of microRNAs which play a central role in cytokine metabolism and regulation. Pro-fibrotic microRNAs were strongly downregulated as compared to anti-fibrotic microRNAs, which showed relatively equal numbers of over- and under-expressed microRNAs. Angiogenic microRNAs were equally over- and under-expressed. Innate- and adaptive immunity microRNAs showed a minimal number of over- and under-expression.

This study reveals expression profiles of microRNAs in human fxH, linked to key processes in fracture healing. Further research will focus on more extensive analysis of these array data and validation of specific microRNA's in different subgroups of patients. Also targets of the miRNAs will be elucidated. These data will broaden our view on potential therapeutic implications of miRNAs in fracture healing.

Abstract **#1962**

DEXAMETHASONE REGULATES CIRCULAR RNA EXPRESSION DURING HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS DIFFERENTIATION

Elena Della Della Bella[P][1], Ursula Menzel[1], Valentina Basoli[1], Céline Tourbier[1,2], Mauro Alini[1], Martin J. Stoddart[1,2]

[1] *AO Research Institute Davos, Switzerland*

[2] *Department of Oral and Maxillofacial Surgery, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany*

Corresponding author's email: elena.dellabella@aofoundation.org

Understanding the molecular mechanisms underlying in vitro differentiation of human bone marrow mesenchymal stromal cells (MSCs) is of utmost importance for regenerative medicine. The role of circular RNAs (circRNA) in gene expression regulation was recently acknowledged, but their effect in stem cell differentiation is currently unclear. The aim of the present study was to identify the differential expression of circRNA in early differentiation of human MSCs. Osteogenic and chondrogenic differentiation were induced in monolayer and pellet cultures, respectively, for 7 days, then samples were collected for RNA isolation. RNA hybridization arrays were used to identify differential circRNA expression during differentiation, and results were validated by qPCR. Moreover, the effect of dexamethasone or the glucocorticoid receptor agonist (+)-ZK216348 on gene expression in monolayer or pellet cultures of undifferentiated cells was assessed. Bioinformatic analysis identified several differentially expressed circRNA during differentiation. Among others, four different circRNAs derived from FKBP5 gene were upregulated in both osteo- and chondrogenesis. Validation experiments showed that total and circular FKBP5 (of which the linear transcripts encode for a co-chaperone of glucocorticoid receptor) were consistently upregulated in both osteogenic and chondrogenic differentiation. Dexamethasone alone was shown to strongly influence the expression of total FKBP5 expression. This effect was mediated by a transactivation mechanism, since (+)-ZK216348, which preferentially induces transrepression, did not have the same influence on FKBP5 gene expression. The functional role of FKBP5-derived transcripts in differentiation should be explored in follow on studies, allowing to better understand the role of dexamethasone during in vitro differentiation.

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Abstract **#1963**

HEPARINIZED ALGINATE BIOMATERIALS FOR THE TREATMENT OF ISCHEMIC INJURIES

Fabiola Munarin[P][1], Isobel Rountree[1], Collin Polucha[1], Kareen LK Coulombe[1]

[1] *Brown University*

Corresponding author's email: fabiola_munarin@brown.edu

In this work we propose an acellular approach to localize and control the release of angiogenic growth factors, for promoting angiogenesis in ischemic injuries. Heparin moieties are bound to alginate backbone (200 μg heparin/mg of alginate), to mimic the natural affinity of the extracellular matrix for heparin-binding growth factors, including bovine serum albumin (BSA), vascular endothelial growth factor (VEGF) and pleiotrophin (PTN). Release profiles of fluorescently-labeled BSA delivered from optimized heparin-alginate hydrogels show a controlled release of the protein over a period of 14 days in vitro and 7 days in vivo. Furthermore, VEGF or PTN loaded in heparinized alginate microspheres or bulk hydrogels enhance angiogenesis in vitro (Matrigel network formation assay performed with translational hiPSC-derived endothelial cells (hiPSC-EC)), ex vivo (aortic ring assay) and in vivo (Matrigel plug assay and subcutaneous implant of heparin-alginate hydrogels). Angiogenic metrics, including endothelial cell migration, number of networks and number of branches, increased to a varying extent comparing the growth-factors loaded heparin-alginate hydrogels with the unloaded and culture medium controls in hiPSC-EC cultures and in the aortic ring assay[1]. Histological assessment of heparin-alginate subcutaneous explants demonstrates a rapid migration of host endothelial cells and macrophages at 4 days after implantation, and the formation of structures resembling the healthy granulation tissue, as assessed by CD68 and CD31 staining (labeling the infiltrating macrophages and endothelial cells, respectively)[2]. Ongoing studies are further investigating this novel therapeutic for pursuing increased vascular density for efficient regeneration of ischemic tissues, including ischemic skin flap wounds and the infarcted myocardium.

Keywords

heparin; growth factors; ischemic tissues

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Abstract **#1964**

POTENTIAL USE OF DECELLULARISED SAPHENOUS VEIN FOR CORONARY ARTERIAL GRAFTING: PRELIMINARY IN VIVO STUDY

Nadiah Sulaiman[P][1,2], Andrew R Bond[2], Vito D Bruno[2], M-Saadeh Suleiman[2], Sarah J George[2], Raimondo Ascione[2]

[1] Center for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia

[2] Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol Royal Infirmary, Bristol, BS2 8HW, UK

Corresponding author's email: nadiahsulaiman@ukm.edu.my

Background and Aims: Autologous grafts, i.e. saphenous vein (SV), and internal mammary artery, are currently utilised in coronary bypass grafting (CABG). Unfortunately, these grafts are limited by the quality, size, and availability from the donor [1]. Bypasses commonly fail because of additional blockages from thrombosis and the irregular performance of vein grafts. Saphenous vein grafts have a 50% failure rate after 10 years [2]. We hypothesised that cells populating the SV are the contributing factor for its lower patency due to their different phenotypic expression. Therefore, this study aims to assess the suitability of decellularised human SV for arterial grafting via in vivo assessment using a porcine model of carotid artery replacement.

Methods: Human SV were decellularised and then implanted in female Landrace pigs (n=3), under strict clinical standards including anti-platelet therapy and anticoagulation with heparin. Pigs were recovered and maintained under optimal animal welfare conditions for 4 weeks. Implanted grafts were surgically exposed and evaluated after a month. Implants were excised, and formalin fixed, along with a section of the native carotid artery, and sectioned at 5 µm thickness. Sections were stained with H&E, Picrosirius Red, EVG and Alcian blue to analyse conduit lumen diameter, wall thickness, and extracellular matrix composition.

Results: In vivo xeno-transplantation confirmed excellent mechanical strength and biocompatibility without mechanical failure, and a 50% patency rate at 4-weeks.

Conclusions: Our preliminary findings support feasibility testing in pig, and the approach has great potential both as a pre-clinical graft testing model, and in the clinical setting.

Keywords

CABG; In vivo study; Decellularised Saphenous Vein

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Abstract **#1965**

CHARACTERISATION OF DENUDED HUMAN UMBILICAL ARTERY: TOWARDS FABRICATING AN EX VIVO INTIMAL HYPERPLASIA MODEL

Siti Sarah Azman[P][1,2], Nadiah Sulaiman[1], Nur Azurah Abdul Ghani[3], Muhammad Dain Yazid[1]

[1] *Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia*

[2] *Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, Tapah Road, 35400 Perak, MALAYSIA*

[3] *Department of Obstetric & Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia*

Corresponding author's email: sarahazman@uitm.edu.my

Vascular endothelium is an important layer that serves as a permeable barrier and helps in the regulation of blood flow. Damage to the endothelial layer can result in the development of intimal hyperplasia (IH). Subsequent event following EC injury that leads to IH development are yet to be fully elucidated. An ex vivo model of intimal hyperplasia is of value to further understand reendothelialisation mechanism in order to combat IH. Healthy human umbilical arteries (hUAs) that are easily obtained will be utilised to set up an ex vivo IH model. In this study, we aim to create and optimise denudation method of hUA, to enable understanding of IH development and reendohelialisation. Here, we assessed the denudation of human umbilical artery (hUA) using Trypsin enzyme with different incubation times applied to the hUAs. The denuded hUAs were stained with Alcian Blue staining, Haematoxylin & Eosin staining and EC coverage and glycosaminoglycans (GAG) coverage were analysed. Results from the study shows that EC coverage in the denuded hUA is reduced as compared to native hUA. While several differences were recorded between denuded and native hUA on the lumen size and GAG distribution. In conclusion, incubation with Trypsin for 10 minutes managed to remove almost 50% of the ECs. Other parameters need to be adjusted to establish a protocol that can result in total hUA denudation. Understanding the potential reendothelialisation of denuded human umbilical artery using recipient's progenitor cells could have a significant impact on finding an alternative vessel graft for bypass surgery.

Abstract **#1966**

ALL CELLS ARE IMPORTANT: MSC AND HUVEC COMMUNICATION IN THE 2D CO-CULTURE MODEL

Irina Beloglazova[P][1], Ekaterina Zubkova[1], Konstantin Dergilev[1], Elizaveta Ratner[1], Yelena Parfyonova[1,2]

[1] *National Medical Research Center for Cardiology, Moscow, Russia*

[2] *Lomonosov Moscow State University, Moscow, Russia*

Corresponding author's email: irene.beloglazova@gmail.com

In this study we used a 2D model of human umbilical vein endothelial cells (HUVEC) contact co-culturing with human adipose stromal cells (MSC) without exogenous matrix addition. As a control we used non-contact co-culturing in Transwell. In the contact model HUVEC self-assembled into the capillary like network (CLN) within 48h in contrast to Transwell. By the first day of contact co-culture, MSC formed a fibronectin scaffold as a basis for extracellular matrix (EM) formation. By the second day both MSC and HUVEC upgraded EM by laminin, collagen 1 and 4. Notably, laminin (mainly laminin 411, according to qRT-PCR data) formed basal-like membrane in the area of contact between MSC and HUVEC and separated them.

We discovered that co-culturing of HUVEC with MSC led to the b3 integrin mRNA upregulation in both cell types and didn't influence mRNA expression of av, b1 and b5 integrins. Blocking antibodies to av integrin subunit not only prevented CLN formation in co-culture but also altered mRNA expression of VEGF receptors, Notch signaling and proteolytic system components in HUVEC.

We can conclude that both MSC and HUVEC are involved in the formation of the specific microenvironment that promotes the CLN formation. Integrin avb3 probably serves as a switch of VEGFRs, Notch and EM signals to control the CLN.

Our results may have important implications in the optimization of cell-based strategies to promote angiogenesis during tissue reparation.

This work was supported by the grants from the Russian Foundation for Basic Research 19-015-00511 and Russian Science Foundation 17-15-01368П.

Keywords

extracellular matrix; angiogenesis; co-culture

Abstract **#1967**

PRECLINICAL EVALUATION OF A NOVEL OSTEOCHONDRAL SCAFFOLD SHOWED ENHANCED BONE AND CARTILAGE REGENERATION

Maryam Tamaddon[1], Gordon Blunn[2], Chaozong Liu[P1]

[1] *UCL, Division of Surgery and Interventional Science, Institute of Orthopaedics*

[2] *School of Pharmacy and Biomedical Sciences,University of Portsmouth, Portsmouth, PO1 2DT, UK*

Corresponding author's email: m.tamaddon@ucl.ac.uk

INTRODUCTION: Treatment of large osteochondral defects presents an unmet clinical need in orthopaedics. This study investigated the efficacy of a multi-layered osteochondral scaffold for repair of large defects in vivo using sheep condyle model. In addition, the effect of bone marrow concentrate (BMC) as a source of growth factors and stem cells1 was evaluated in conjunction with the osteochondral scaffold. METHODS: The multi-layered scaffold was fabricated using additive manufacturing techniques. A collagen/hydroxyapatite scaffold was used as control. 24 sheep were randomly assigned to one of the four treatment groups: scaffold ± BMC and control ± BMC. The tissue was retrieved 6 months post-operation. Bone regeneration was evaluated using µCT, while cartilage regeneration and quality were examined macroscopically (modified ICRS) and by histology and gene expression. Gait was examined pre-operation and before termination.RESULTS: Gross evaluations of the joint showed minimal to slight reactions for all groups. Regenerated cartilage was not macroscopically different between the groups, however, a significant upregulation of mRNA for type-II collagen showed an enhance cartilage quality with the novel scaffold. µCT data revealed that the bone ingrowth was higher in the scaffold group, while bone voids remained in the control groups. Gait parameters were not affected by the choice of the treatment.CONCLUSIONS: Collectively, these data illustrate that the novel scaffold support bone ingrowth and a hyaline-like cartilage formation. Our results indicated that the addition of BMC to scaffold has some potentially beneficial effects on tissue regeneration but not on the functional endpoint of orthopaedic interest.

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Abstract **#1968**

3D PRINTED POLYCAPROLACTONE/BIOACTIVE GLASS/TRICALCIUM PHOSPHATE COMPOSITE SCAFFOLDS: EFFECT OF MATERIAL FORMULATION ON THE PRINTABILITY OF INK AND PHYSICOCHEMICAL PROPERTIES OF FABRICATED SCAFFOLDS

Seyed Ali Poursamar[1], Hasti Esmaeili[2], Mansure Kazemi[3], Mehdi Mehdikhani[2], Mohammad Rafienia[4]

[1] *Biomaterials, Nanotechnology, and Tissue Engineering Group, Isfahan University of Medical Sciences, Advanced Medical Technology Department, Isfahan 81746-73461, Iran*

[2] *Department of Biomedical Engineering, Faculty of Engineering, University of Isfahan, Isfahan, Iran*

[3] *Department of Applied Cell Sciences, Faculty of Medicine, Kashan University of Science, Kashan, Iran*

[4] *Biosensor Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

Corresponding author's email: alipoursamar@gmail.com

Bone tissue engineering presents a promising strategy for the reconstruction of large bone defects which still remains challenging for clinicians. Thanks to advances in technology and the development of new scaffold fabrication methods including three-dimensional printing, the ability to create constructs with controllable characteristics is provided.[1,2,3] The aim of this study was to investigate the potentiality of Extrusion-based 3D-printing (EBP) to fabricate polymer-ceramic composite bone substitutes. Using Polycaprolactone, 45S5 bioactive-glass (BG), and β-Tricalcium Phosphate (β-TCP) as raw materials, PCL/BG/TCP scaffolds with different ratio of BG/TCP (10/90, 70/30, and 90/10) were prepared by EBP. The effects of ceramic ratio on the rheology of printing ink and physicochemical properties of scaffolds were evaluated. Results showed that all compositions present a shear-thinning behavior making them appropriate for additive manufacturing. The higher percentage of β-TCP leads to a sticky state in the printing ink that is associated with reduced ink printability. Increasing the BG content decreased this fault and improved print performance. This observation can be assigned to the impact of the surface charge interaction of BG and β-TCP particles. Scanning electron microscopy observations revealed that different ink formulations could form a pre-designed interconnected porous construct, though 90/10 BG/TCP scaffold pores showed more uniform structure and approximately rectangular shape. However, increasing the BG percentage was associated with a slight decrease in mechanical properties. Finally, optimization of the composite formulation allowed controlling the morphology, porosity, and mechanical properties of the final scaffolds, and BG/TCP: 90/10 could be a suitable composition in preparation of bone scaffold.

Keywords

3D Printing; Bioactive Glass; Tricalcium Phosphate

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Abstract **#1969**

DESIGN AND PREPARATION OF 3D-PRINTED MEDICAL DEVICES TO PREVENT AND TREAT BIOMATERIAL-ASSOCIATED INFECTIONS

David Martinez Perez[P][1]

[1] *Warsaw University of Technology*

Corresponding author's email: david.perez@pw.edu.pl

Modifying the surface of medical devices with antimicrobial coatings is one of the most effective approaches to prevent biomaterial-associated infections (BAIs).

Polymers are commonly used as antimicrobial coatings as they can be easily synthesized and loaded with different drugs. Generally, polylactic acid (PLA) and poly(lactic-co-glycolic) acid (PLGA) are commonly used for drug release due to the high biocompatibility and FDA approval for drug delivery applications.

Drop on demand (DOD) technology involves data-driven and non-contact techniques that enable high speed and precise deposition of nanoliter volumes of material on a specific target.

In this work we propose a novel approach, using a drop on demand 3D printing technique for the development of antimicrobial polymeric coatings on medical devices, such as titanium orthopaedic implants (commercial and 3D printed by SLM) and low-density polyethylene catheters.

The DOD coatings were developed by using a commercial 3D Printer (3DDiscovery, regenHU, Switzerland). A home-made stepper motor rotating device was designed and developed for coating the LDPE catheters and SLM Ti implants.

Ink formulations based on PDL or PLGA were developed in mixtures of different organic solvents, incorporating different antimicrobial compounds, such as rifampicin, niclosamide, and SAAP-148 (LL-37 derivative).

The printability and viscosity (DV-III Ultra, Brookfield, USA) of the formulations were studied at different polymer concentrations.

Experimental results show a small printability window of the ink viscosities, from 7 to 24 mPa-s.

The antimicrobial evaluation of PDL-PEG-Niclosamide coated catheters showed a 4 and 4.9 log reduction in biofilm formation on S.aureus. in vitro and in vivo respectively.

Keywords

3D Printing; Antimicrobial; Medical devices

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Abstract **#1970**

ENGINEERED LAMININ-BINDING EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STEM CELLS FOR PERIPHERAL NERVE REGENERATION

Mai Quyen Nguyen[1,2], Madhusudhan Reddy Bobbili[1,2,3], Johannes Grillari[1,2,3], David Hercher[1,2]

[1] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Donauschingenstraße 13, 1020 Vienna, Austria*

[2] *Austrian Cluster for Tissue Regeneration*

[3] *University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria*

Corresponding author's email: mai.quyen.nguyen@trauma.lbg.ac.at

Peripheral nerve injuries often result in sensory and motor dysfunction in respective parts of the body. So far, peripheral nerve regeneration is often associated with poor functional recovery. Important facilitators of the regeneration process are Schwann cells (SCs), which basement membrane is chiefly comprised of laminin. Extracellular vesicles (EVs) are considered important for intercellular communication and transfer of biological information. Mesenchymal stem cell-derived EVs (MSC-EVs) have been identified as a new therapeutic option due to their function as a drug delivery system. However, the precise delivery of EVs to the site of interest upon administration remains challenging. To overcome this issue, overexpressed EV surface marker protein CD81, from the tetraspanin protein family, has been modified toward preferential binding of laminin.

This study was designed to achieve production of laminin-binding EVs derived from MSC by modification of the large extracellular loop (LEL) of CD81.

Specific CD81-LEL sequences are cloned into lentiviral vectors encoding the expression cassette for full-length CD81 proteins fused with eGFP or firefly luciferase under the control of human cytomegalovirus (CMV) promoter. Stable cell lines are obtained upon transformation of Wharton's Jelly MSCs and selected by sorting for high expressers. MSC-EVs are further isolated by ultracentrifugation and characterized by nanoparticle tracking analysis, flow cytometry and western blot.

Our results demonstrate the feasibility of production of designed laminin-binding EVs derived from MCSs. This study represents the basis for further investigation on EVs regarding their targeted binding to laminin, their internalization by SCs and their influence on peripheral nerve regeneration processes.

Abstract **#1971**

DEVELOPMENT OF NEW GENERATION HYDROCOLLOID BIO-INK FOR 3D BIOPRINTING

Ahu Arslan Yildiz[P][1]

[1] *Izmir Institute of Technology, Department of Bioengineering*

Corresponding author's email: arslanahu@gmail.com

Bioprinting enables the production of 3-dimensional (3D) structures by combining bioinks, living cells, extracellular matrix (ECM) components, biochemical factors, proteins, drugs; and it has recently become one of the most promising techniques in the field of tissue engineering. The successful production of the 3D structure to be created by 3D bioprinting technology depends on the properties of the bio-ink to be used. Hydrogel/hydrocolloid materials used as bio-inks are developed using synthetic and natural polymers where they have the necessary rheological properties for printing, they also have biocompatibility, low toxicity and support for cell attachment. Natural hydrogels, which have the ability to mimic the extracellular matrix structure and function at a high rate, are highly preferred bioink materials for bioprinting applications. Polysaccharide-based hydrogel/hydrocolloids are one of the largest subclasses of natural polymers and are commonly used in food industry, drug release and tissue engineering applications with their gelling and biocompatibility properties. Hydrocolloids obtained from the seeds of some plants are among the promising natural materials in tissue engineering applications and the development of new generation bio-inks with their high water holding capacity, anti-inflammatory, and antioxidant properties. Here we report development of a new generation polysaccharide-based bio-ink for 3D bioprinting applications. The bioink was obtained through "from waste to the bench-top" approach by utilizing quince seed as a raw material. It was shown that developed bioink demonstrates desirable properties including viscoelasticity and processability, biocompatibility and non-toxicity, as well easy to obtain and cost effective as a bioink.

Keywords

3D Bioprinting; polysaccharide bio-ink; 3D cell culture

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Abstract **#1972**

T CELL ACTIVATION DIRECTS ARTICULAR REPAIR OF FULL-THICKNESS OSTEOCHONDRAL DEFECTS IN RAT

Johanna Bolander$_P$[1,2], Cara Clouse[1], Tim Herpelinck[3], AnnaLisa Wilson[1], Gurstavo Moviglia[1], Frank Marini[1], Anthony Atala[1]

[1] Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, United States of America

[2] Tissue Engineering Laboratory, Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium.

[3] Tissue Engineering Laboratory, Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium.nstitute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, United States of America

Corresponding author's email: jbolande@wakehealth.edu

Osteochondral defects in the adult human fail to heal, resulting osteoarthritis (OA). The onset of OA has been linked to a prolonged pro-inflammatory response induced by the injury or repetitive microtrauma. This may be caused by an imbalance in the signalling cascades during the transition from the pro-inflammatory to the pro-regenerative phase.

Small (Ø: 0.15mm, SD) or large (Ø: 1.5mm, LD) full-thickness osteochondral defects were created in the trochlear groove of 10-week old wild type (T-cell+) or T cell deficient (T-cell-) female rats. Healing was characterized up to twelve weeks. Pathological evaluation confirmed that the T-cell+-SD model displayed functional healing (OARSI score 0/4), T-cell+-LD resulted in moderate fibrosis (2/4) and Tcell--SD and Tcell--LD displayed mild (1/4) and severe (3/4) fibrosis, respectively. Analysis at one week confirmed a corresponding trend between the healing potential to extracellular matrix (ECM) production, progenitor- and inflammatory cell activation. Interestingly, scRNAseq confirmed a unique inflammatory-progenitor cell population present within the defect area in the Tcell+-SD model. Injection of in vitro cartilage-activated lymphocytes, placenta derived progenitor cells (PLCs) or a 24h co-cultured combination thereof 1 week post defect creation improved the healing. But only co-cultured cells completely regenerated the Tcell--SD and LD defects based on OARSI scoring and lubricin secretion.

These results confirm the integral role of the balanced activation of lymphocytes and progenitor cells for functional osteochondral regeneration. Furthermore, the presented findings show effective, stable articular cartilage regeneration after combined treatment of cartilage-activated lymphocytes and PLCs in moderate to large osteochondral defects.

Keywords

Regeneration; Osteoarthritis; Cell therapy

Abstract **#1973**

BIOFABRICATION BY MAGNETIC LEVITATIONAL ASSEMBLY OF CELLS INTO DEFINED 3D CELLULAR STRUCTURES

Ahu Arslan Yildiz$_P$[1]

[1] *Izmir Institute of Technology, De*

Corresponding author's email: arslanahu@gmail.com

In the field of tissue engineering 3D (three dimensional) cell culture studies have increased over the years since they are the closest models of real tissues. Compared to the 2D models, there is a big improvement on cell growth, morphology, differentiation, gene and protein expression when 3D system is utilized. Because of these advantages 3D cell culture is commonly used for tissue engineering, artificial organ technologies, regenerative medicine, drug development, drug screening and stem cell studies. Despite promising advances in these areas, there are still unmet needs to completely fulfill all requirements. Sophisticated tools, methodologies and materials are still required for further development in tissue engineering; especially for cellular assembly, single cell level control, easy control over biofabrication system, direct forward cellular imaging and analysis. Recently, magnetic levitation technology that overcomes most of the above mentioned problems, has been utilized for the formation of 3D cellular structures. Magnetic levitational assembly of cells provide rapid, simple, cost-effective 3D cell culture formation while ensuring scaffold-free microenvironment.
This contribution summarizes our efforts in designing new setups and assembling cellular entities via contactless magnetic manipulation as functional biological units for 3D cell culture and tissue engineering. It has been demonstrated that especially cell-cell interactions are favored by utilizing magnetic levitational assembly. With the developed technology, employed models also provide us possibility to adapt several components into different platforms for further tissue engineering applications.

Keywords

Magnetic Levitation; Cellular Assembly; 3D cell culture

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Abstract **#1974**

DYNAMIC BIOFUNCTIONALIZATION AND MODULATION OF HYDROGELS BY PEPTIDE-FOLDING MEDIATED INTERACTIONS AND BIOORTHOGONAL CROSSLINKING FOR 4D BIOPRINTING

Sajjad Naeimipour$_P$[1], Robert Selegård[1], Daniel Aili[1]

[1] *Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, 581 83, Linköping, Sweden*

Corresponding author's email: sajjad.naeimipour@liu.se

3D bioprinting is a promising method for generating cell- and tissue models and constructs for drug development, tissue engineering, and fundamental studies of cell-matrix interactions. The properties of the bioinks, comprising biomaterials and cells, determine printability, cell viability, and post-printing cell survival and behavior. Most current hydrogel-based bioinks provide limited possibilities to tune and alter the generated structures' properties after printing. Here, we describe a novel hyaluronan/poly(ethylene glycol) based hydrogel system for bioprinting that combines robust and tunable covalent bioorthogonal cross-linking strategy [1] with peptide-folding mediated interactions to enable dynamic modulation of hydrogel properties [1,2]. The peptides were de novo designed to homo- or heterodimerize and fold into a helix-loop-helix motif and dimerize into four-helix bundles. Peptide dimerization enabled dynamic modulation of cross-linking density and hydrogel functionality before, during, and after printing[2]. A toolbox of functionalized peptides with different functionalities was developed, which allowed for altering cell adhesion and enhancing retention of biofunctional enzymes. The latter was demonstrated by triggering enzyme-mediated biomineralization of the printed structures [1]. This flexible strategy for controlling and changing hydrogel properties can facilitate the development of 4D bioprinting techniques.

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Abstract **#1975**

EVALUATION OF ELASTOMERIC POLYMERS FOR MYOBLAST SURVIVAL, PROLIFERATION AND DIFFERENTIATION

Lisanne Terrie[P][1], Maarten Mees[2], Pieter De Wever[2], Melanie Gerard[1], Wim Thielemans[2], Lieven Thorrez[1]

[1] *Tissue Engineering Lab, Dep. Development and Regeneration, KU Leuven Kulak*

[2] *Renewable Materials and Nanotechnology Research Group, Dep. Chemical Engineering, KU Leuven Kulak*

Corresponding author's email: lisanne.terrie@kuleuven.be

For developing tissue-engineered skeletal muscle three-dimensional scaffolds may provide a physical support. Among a variety of biomaterials developed and used for soft tissue engineering, elastomeric scaffolds remain promising due to their compatibility with the elastic properties of the host tissue. Elasticity, which implies recovery from deformation, is of particular importance in the context of dynamic tissues like muscle. We evaluated porous elastomeric 3D scaffolds from the family of poly(polyol sebacate) for survival, proliferation and differentiation of murine and human myoblasts. This family of soft, biodegradable chemically cross-linked elastomers are biocompatible and inexpensive [1]. They have already been studied in cardiac [2], [3] and vascular tissue engineering (reviewed in [4]) but not yet in skeletal muscle tissue engineering. Several compositions of elastomers were evaluated with alamar blue and live/dead assay for myoblast survival over 6 days and based on these results, PGS 1:1 was chosen for further differentiation analysis. Preliminary data on myoblast differentiation capacity when integrated in the PGS 1:1 scaffold was generated using human myoblasts. From confocal imaging performed after staining for the differentiation marker tropomyosin, we observed efficient differentiation into myofibers throughout the scaffold. PGS 1:1 is a suitable, biocompatible scaffold for skeletal muscle tissue engineering allowing for myofiber formation.

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Abstract **#1977**

INTRAVASCULAR INFUSIBLE EXTRACELLULAR MATRIX MODULATES INFLAMMATORY RESPONSE IN MOUSE MODEL OF SYSTEMIC INFLAMMATION

Anne Lyons[P][1], Raymond Wang[1], Ryan Middleton[1], Mark Hepokoski[1], Karen Christman[1]
[1] *University of California San Diego, La Jolla, California, United States*
Corresponding author's email: anlyons@ucsd.edu

Introduction: Systemic inflammatory conditions (e.g. sepsis and severe viral infections like COVID-19) are characterized by an overwhelming innate immune response that leads to multi-organ failure [1]. Decellularized extracellular matrices (ECM) have previously demonstrated pro-regenerative properties through modulation of the immune response [2]. Infusible ECM (iECM) was developed for systemic delivery, targeting and treating sites of vascular injury. We hypothesized iECM delivery would dampen the systemic inflammatory response in a lipopolysaccharide (LPS) mouse model.

Methods: iECM was prepared from decellularized porcine left ventricle based on previous protocols [3]. C57BL6/J mice underwent dual intraperitoneal LPS injection and then tail vein injection of saline or iECM (10 mg/mL). Thirty hours post-LPS dose, mice were euthanized and heart, lungs, brain, kidneys, spleen, and liver were harvested (n = 6 mice/group). Tissues were processed for gene expression by qRT-PCR and Nanostring nCounter® Immunology Panels, immune cell identification by flow cytometry, and cytokines by LegendPlex® Mouse Inflammation Panels.

Results: qRT-PCR identified significant downregulation of Il1b and Il6 across multiple tissue types in iECM vs. saline-treated mice. Nanostring transcriptomic analysis confirmed downregulation of multiple inflammatory cytokines and chemokines. IL-6 cytokine expression was significantly reduced across multiple organs along with IL-1α and IFN-γ in the lungs, and IL-1β and IL-17A in the spleen.

Discussion: Results demonstrated iECM dampens the systemic inflammatory response to LPS, indicating its potential for treating conditions such as sepsis and COVID-19 pathology.

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Abstract **#1978**

AMELIORATION OF DIABETIC WOUND USING MENSTRUAL BLOOD STEM CELLS SEEDED ON NANOFIBROUS BILAYER SCAFFOLD COMPOSED OF AMNIOTIC MEMBRANE AND SILK FIBROIN IN MOUSE MODEL

Ebrahim Mirzadegan[P][1], Zahra Saffarian[2], Hannaneh Golshahi[2], Maryam Darzi[2], Somayeh Khorasani[2], Somaieh Kazemnejad[2]

[1] *Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*

[2] *Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*

Corresponding author's email: e.mirzadegan@avicenna.ac.ir

To explore noticeable immunomodulatory activity, angiogenic potential, and regenerative effects of menstrual blood-derived mesenchymal stem cells (MenSCs) in combination with the fabricated bilayer scaffold (bSC) in the healing of diabetic wound, here we show the effect of MenSCs-seeded on bSC in the treatment of diabetic wound created in the dorsal dermis of type-1 diabetic model of C57BL/6 mice was induced by streptozotocin in comparison to control treatments. The control groups included no treatment (NT), amniotic membrane treatment (AM), bSC treatment, foreskin-derived keratinocytes/fibroblasts seeded on bSC treatment (FK+bSC). Wound closure was inhibited by fixing a circular silicone splint to the skin to prevent rapid contracture of the wounds and simulate re-epithelialization. Wound healing evaluations in the diabetic mice model were studied at 3, 7 and 14 days after their treatment. On day 14 in the MenSCs+bSC group, the gross morphology, epithelialization, and histological changes in regenerated wound tissues showed the most similarity to intact skin. Moreover, the wound healing was typically faster in the MenSCs+bSC group compared to other diabetic groups. Immunofluorescence staining of mouse skin depicted higher levels of CD31 and von Willebrand factor (VWF), a higher ratio of M2/M1 macrophages, higher expression of involucrin as keratinocyte marker, and higher levels of the neural marker in the MenSCs+bSC group in comparison with other treatment groups. The dramatic effects of MenSCs as a promising adult stem cell population accompanied by the significant impact of fabricated bilayer scaffold sound to mediate a promoting and appropriate circumstance on wound healing and skin regeneration.

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Abstract **#1979**

VASCULARISING DEVELOPING BONE ORGANOIDS ON A CHIP TO MODEL ENDOCHONDRAL OSSIFICATION AND FRACTURE HEALING

Ian T Whelan[P][1], Ross Burdis[1], Emad Moeendarbary[2], David A Hoey[1], Daniel J Kelly[1]

[1] *Trinity College Dublin*

[2] *University College London*

Corresponding author's email: iwhelan@tcd.ie

Long bones develop and regenerate through the process of endochondral ossification (EO); the replacement of a cartilage template with new bone. Dysregulation of EO can result in conditions such as fracture non-unions, chondrodysplasias and osteochondrosis. In addition, mimicking the process of EO is central to many next generation tissue engineering strategies to heal bone defects. Critical in EO is the interaction between cartilage and vasculature, yet our understanding of this essential interaction is limited (1). To address this problem, we have developed an advanced in vitro model of EO to study bone development and pathophysiology on a chip. To enable this, we introduce developing human bone organoids (engineered cartilage at different stages of maturation) into microfluidic devices containing perfusable vascular networks, with the goal of recreating and better understanding the complex interaction between developing bone and the vasculature during EO. The model is capable of predicting a number of key features of EO, including a cartilage phenotype dependent effect on vasculature. Specifically, we find that mature cartilage inhibits vascular invasion, which correlates with an upregulation of cartilage specific anti-angiogenic genes. Additionally, we observe upregulation of the pluripotency genes SOX2 and OCT4 in the presence of vasculature, providing support for the hypothesis of chondrocyte transdifferentiation during fracture healing (2). Predictive models of EO are fundamental to advancing our understanding of human bone (patho)physiology, and this model represents an advanced in vitro system to probe the mechanisms that underpin this essential process.

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Abstract **#1980**

BIOMECHANICAL ANALYSIS OF NANOSTRUCTURED CALCIUM PHOSPHATE COATED PEEK AND COATED CF-PEEK IMPLANTS

Leena Jongpaiboonkit$_P$[1], Jeffrey Badura[1]

[1] *Medtronic*

Corresponding author's email: leena.jongpaiboonkit@medtronic.com

Introduction: Nanostructured calcium phosphate (CaP) coatings have the potential to produce osteostimulatory, bone forming characteristics on the surface of PEEK implants. This biomechanical study examines: 1) adhesion of a CaP coating to PEEK and Carbon Fiber Reinforced (CF) PEEK substrates, and 2) the impact of surface pre-treatment on the mechanical properties of both types of PEEK.

Methods: A coating was applied to PEEK and CF-PEEK cylinders through a proprietary coating process. Materials were subjected to surface pre-treatments and subsequently incubated in a pH adjusted coating solution for 4 days, resulting in a continuous, plate-like nanostructure coating. Implant insertion was replicated using Sawbones block 20PCF and controlled insertion torque. Coating adhesion on each substrate was then qualitatively assessed by SEM and Alizarin staining. A standard mechanical test was conducted to identify any difference in yield strength and compression modulus between coated and uncoated samples.

Results: Overall, insertion into Sawbones did not cause coating delamination, except where uneven insertion caused shearing of both the coating and the underlying PEEK under high torque. The compression tests demonstrated no significant change in compression modulus or yield strength between coated and uncoated samples.

Conclusion: These results suggest that this coating process may be used to apply calcium phosphate coatings to both forms of PEEK with adhesion characteristics that survive implant insertion torque. Furthermore, this coating process does not appear to have a detrimental effect on the mechanical properties of PEEK or CF-PEEK.

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Abstract **#1981**

THE TRANSCRIPTIONAL LANDSCAPE OF CANINE NOTOCHORDAL CELL RICH-DISCS: FROM HEALTH TO DISEASE

Deepani Poramba-Liyanage$_P$[1], Frank Riemers[1], Xiaole Tong[1], Vivian Tam[2], Anne Camus[3,4], Danny Chan[2], Marianna Tryfonidou[1]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, 3584 CM, Utrecht, the Netherlands

[2] School of Biomedical Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China.

[3] INSERM, UMR 1229, RMeS, Regenerative Medicine and Skeleton, Université de Nantes, ONIRIS, Nantes F-44042, France.

[4] Université de Nantes, UFR Odontologie, Nantes F-44042, France

Corresponding author's email: d.w.l.porambaliyanage@uu.nl

Intervertebral disc degeneration (IVDD) is a key public health issue and the leading cause of lower back pain1. IVDD is often associated with altered cellularity, absence of vacuolated nucleus pulposus cells (NPCs), and appearance of nested cells within the nucleus pulposus (NP) at the centre of the IVD2,3. Repopulating the degenerated IVD by regenerative NPCs for biological repair and restoration of disc function is extensively studied as an advanced therapy for IVDD within the iPSpine project. We sought to understand the cellular transition within the NP using single cell transcriptomics in canine NPCs. Dogs provide an informative species to conduct comparative analysis; both humans and dogs share the cellular changes within NP leading to IVDD during maturation and ageing3. To obtain in-depth knowledge of the NPC signature, we assess single cells from (a) canine primary NPCs and mature NPCs from healthy discs, and (b) cells that reside within the degenerated NP from canine patients and (c) NPCs from chondrodystrophic dogs that have a predisposition for IVDD. We present a simple strategy for obtaining single cells from NP tissue using enzymatic digestion and EDTA treatment. Cells are then subjected to Sort Seq; a single-cell sequencing platform using cell-capture plates and flow cytometry sorting; that makes it possible to analyze transcriptomics of live single-cell populations of interest. Our findings help define the transcriptomic landscapes of NPCs through health and disease. We reveal common and preserved denominators of NP cell fate and highlight the essential instructions for exploitation towards regenerative therapies.

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Abstract **#1983**

EXTRACELLULAR MATRIX OF SENESCENT CELLS: WHAT SHOULD WE EXPECT?

Rita Sá Ferreira$_P$[1,2], Patrícia Pitrez[1,2], Maximilian Quaas[3], Tilo Pompe[3], Alessandro Ori[4], João Passos[5], Lino Ferreira[1,2]

[1] *Center for Neurosciences and Cell Biology, Coimbra, Portugal*

[2] *Faculty of Medicine, University of Coimbra*

[3] *Institute of Biochemistry, Faculty of Life Sciences, Universität Leipzig, 04103 Leipzig, Germany*

[4] *Leibniz Institute on Aging-Fritz Lipmann Institute (FLI), Jena, Germany*

[5] *Mayo Clinic, Rochester, Minnesota, USA*

Corresponding author's email: ritasaferreira15@gmail.com

Ageing is a risk factor for the development of cardiovascular diseases. Many of these diseases are caused or exacerbated by dysfunctional vasculature. The major hallmarks of vascular aging are the stiffening of the vasculature, resulting from changes in the extracellular matrix (ECM) and endothelial dysfunction due to a pro-inflammatory environment, increased oxidative stress and consequent accumulation of senescent cells [1-3]. Cell senescence, an important axis of vascular ageing, is described as a state of irreversible cell cycle arrest and it can be triggered by different factors, such as DNA damage, telomere exhaustion, oncogene activation and oxidative stress [4]. Currently our understanding about the ECM of senescent cells is limited. Here, we have investigated the composition of ECM from senescent smooth muscle cells (SMCs). SMC cultures of proliferative and irradiation-induced senescence were decellularized to obtain the ECM. Atomic force microscopy measurements showed that the decellularized ECM from senescent cells presented a higher Young's modulus than proliferative cells. Characterization of ECM from proliferative and senescent cells by proteomics showed significant differences in their protein content. ECM-related proteins, such as collagens, proteoglycans and fibulins were the mostly downregulated in senescent ECM. On the other hand, cell adhesion and cytoskeleton proteins, such as desmin, vimentin, talin, integrins and cadherins were upregulated in senescent ECM. Overall, our work demonstrates significant differences between ECM derived from senescent or proliferative SMCs.

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Abstract **#1984**

A STUDY ON MYOGENIC COMMITMENT OF HUMAN MESENCHYMAL STEM CELLS BY MYOBLAST CO-CULTURE IN STATIC AND DYNAMIC ENVIRONMENT

Pasqualina Scalaᴘ[1], Rossella Vitolo[1], Carmine Selleri[1], Laura Rehak[2], Nicola Maffulli[1], Giovanna Della Porta[1,3]

[1] *Department of Medicine, Surgery and Dentistry, University of Salerno Via S. Allende, 84081 Baronissi (SA), Italy;*

[2] *Athena srl., R&D Department, Viale Europa 139 - 50126 Firenze (FI)*

[3] *Centro Interdipartimentale BIONAM, Università di Salerno, Via G. Paolo I, Fisciano (SA), Italy*

Corresponding author's email: pscala@unisa.it

Introduction: The development of in vitro model of skeletal muscle regeneration is extremely challenging. Muscle in vitro models often involves the use of mesenchymal stem cells from bone marrow (BM-MSCs) [1], however, optimum medium composition to promote myogenic commitment of stem cells has not yet reported. Despite growth factor mixture and their different concentrations in culture media is still under debate, bFGF was the most used at concentration of 10 ng/mL [2]. Furthermore, several studies reported 3D scaffolds as stem cells culture system and co-culture systems have been also proposed [3,4]. In the present work an in vitro model of myogenic commitment by using BM-MSCs and Myoblast co-culture with bFGF supplemented medium is proposed in both static and dynamic conditions to explore the potential paracrine effect of myoblasts on MSCs differentiation.

Materials and methods: In vitro culture of human Bone Marrow-Mesenchymal Stem cells (hBM-MSCs) and human Myoblasts allowed to study the paracrine effect of muscle cells on stem cells. The system was explored in both dynamic and static environment.

Results and Discussion: hBM-MSCs commitment towards a myogenic phenotype was confirmed both via immuno-histology that revealed Desmin fiber deposition and by q-RT-PCR that indicated the increase of gene expression of myogenic factors as Myf5, MyoG, MyoD, MFR4, Desmin and Myosin heavy chain II. The dynamic co-culture system developed can be used as highly predictive model to study skeletal muscle regeneration.

Keywords

myogenic commitment; perfusion system; 3D scaffold

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Abstract **#1985**

NEW SINGLE-STAGE, ARTHROSCOPIC CARTILAGE REGENERATION THERAPY WITH NASAL CHONDROCYTES

Gyözö Lehoczky$_P$[1,2], Marcus Mumme[3,2,4,5], Raluca Elena Trofin[2], Shikha Chawla[2], Karoliina Pelttari[2], Martin Haug[6], Christian Egloff[3], Marcel Jakob[7], Ivan Martin[2], Andrea Barbero[2]

[1] *1.University Hospital of Basel, Department of Orthopedic Surgery and Traumatology, Basel, Switzerland*

[2] *Department of Biomedicine, Tissue Engineering Laboratory, University of Basel, University Hospital Basel*

[3] *University Hospital of Basel, Department of Orthopedic Surgery and Traumatology*

[4] *SportClinic Zurich Hirslanden*

[5] *University Children's Hospital of Basel*

[6] *University Hospital of Basel, Department of Plastic, Reconstructive and Aesthetic Surgery and Hand Surgery*

[7] *CrossKlinik Basel*

Corresponding author's email: g.lehoczky@usb.ch

Background

Nasal chondrocytes (NC) have higher and more reproducible chondrogenetic capacity than articular chondrocytes, could thus achieve better clinical results, and were previously introduced in a first-in-human study (1.) to proof safety and feasibility.

Our aim was to create a new, single-stage and injectable therapeutic approach using autologous nasal chondrocytes.

Materials & Methods

Fresh nasal septal cartilage grafts were digested according to a new, rapid protocol, yielding "rapid isolated" nasal chondrocytes (p0 NCs). Cell yield, viability and proliferation rate were assessed.

The p0 NCs were either used for in vitro pellet culture, or seeded in PL-PEG gels (polyethylene glycol (PEG) gel enriched with pooled PRP extract – platelet lysate (PL)), in a low cell number, and cultured in vitro. Histological, immunofluorescence and biochemical analyses were carried out at day 0, after 1 and 4 weeks.

Results

Rapid isolated NCs show similar viability (mean 99.0±1.3% vs 98.4±1.6%) and proliferative capacity (mean 0.66±0.14 vs 0.78±0.25 doublings/day) as cells after standard digestion; however, cell yield is even higher after the new protocol. Pellet culture showed good chondrogenetic capacity. In vitro gel cultures showed proliferation of the cells with 6.33 ± 0.51 mean population doublings in 4 weeks. At the meantime, also cartilaginous matrix production was observed, with GAG/DNA ratio of mean 12.3±7.9.

Conclusions

Rapid isolation protocol resulted in viable NCs with good in vitro chondrogenic capacity. The cells, implanted in low-density in PL-PEG gel showed proliferation and chondrogenesis in vitro. This approach can lead to establish a new arthroscopic therapy for cartilage lesions.

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Abstract **#1986**

A STUDY ON HBM-MSCS CHONDROGENIC COMMITMENT BY 3D COLLAGEN SCAFFOLD LOADED WITH PLGA CARRIERS FOR TGF-B1 CONTROLLED RELEASE

Erwin Pavel Lamparelli[P][1], Joseph Lovecchio[2], Rossella Vitolo[1], Emanuele Giordano[2], Carmine Selleri[1], Nicola Maffulli[1], Giovanna Della Porta[1,3]

[1] *Department of Medicine, Surgery and Dentistry, University of Salerno, Via S. Allende, Baronissi (SA), Italy*

[2] *Department of Electrical, Electronic and Information Engineering "Guglielmo Marconi" (DEI), University of Bologna, Via dell'Università 50, Cesena (FC), Italy*

[3] *Centro Interdipartimentale BIONAM, University of Salerno, University of Salerno, Via G. Paolo I, Fisciano (SA), Italy*

Corresponding author's email: elamparelli@unisa.it

Introduction: Treatment of hyaline cartilage lesions remains a major challenge owing to the avascular nature of itself, which prevents spontaneous healing. To date the use of Mesenchymal Stem Cells (MSC) and tissue engineering protocols represents an alternative strategy and offers great potential to improve joint therapy. The development of 3D scaffold able to stimulate the formation of a new cartilaginous tissue is crucial to the success of the therapeutic strategy. Collagen-based scaffolds have been described as appropriate to support the chondrocyte attachment because are more like natural ECM [1,4].

Methods: A three-dimensional (3D) collagen scaffold was assembled as biomimetic extracellular matrix for human Bone Marrow Mesenchymal Stem Cells (hBM-MSC) induction towards chondrogenic phenotype by dispersing poly-lactic-co-glycolic acid (PLGA) microcarriers, carrying and delivering Transforming Growth Factor (hTGF-β1) payload, within the 3D structure. Cultures were performed both in static than dynamic condition (perfusion).

Results: hBM-MSCs early commitment towards a chondrogenic phenotype was confirmed both via immuno-histology that revealed the type II collagen fiber deposition within the scaffold and by q-RT-PCR that indicated the increase of gene expression of SOX9 and COL2A1, after 16 days of culture. The dynamic culture by perfusion assured a better mass exchange and more effective scaffold matrix reshaping.

Discussion and Conclusions: The data suggested the effectiveness of 3D collagen scaffold carrying PLGA microspheres for drug delivery as highly predictive in vitro model and opened perspectives for its use as implantable advanced therapy device to promote cartilage regeneration.

Keywords

3D collagen scaffold; Chondrogenic commitment; Perfusion bioreactor

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Abstract **#1987**

ELECTROSPUN SCAFFOLDS WITH EXPANDED PORES TO SUPPORT CELLULAR INFILTRATION FOR SKIN REGENERATION

Tânia Vieira$_P$[1], Ana Margarida Rebelo[1], João Paulo Borges[1], Célia Henriques[1], Jorge Carvalho Silva[1]
[1] Centro de Investigação de Materiais, Institute for Nanostructures, Nanomodelling and Nanofabrication, CENIMAT-I3N, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
Corresponding author's email: ts.vieira@fct.unl.pt

Tissue engineered skin substitutes have been developed in order to treat full-thickness skin injuries. Several substitutes are available on the market but they do not provide, on their own, full skin regeneration [1]. Therefore, the use of autografts continues to be involved in any treatment of severe wounds.
Electrospun mats have been widely used to create scaffolds for skin replacement due to their structural similarities to the extracellular matrix (ECM) of the tissue, to allow for cellular regeneration [2]. However, electrospun mats have relatively small pores and high packing density of the fibers that prevents cellular infiltration and, consequently, new tissue ingrowth. To overcome this limitation, in this work, two different approaches were used to expand the pores of electrospun mats. In one approach, chitosan (CS) grains were loaded in polycaprolactone (PCL) solution. In another approach, glucose grains with different dimensions (< 50 µm, 50-100 µm and 100-150 µm) and concentrations, loaded in a polyethylene oxide solution, were co-electrospun with polylactic acid (PLA) and leached out using distilled water. All scaffolds presented pores large enough to facilitate the infiltration of fibroblasts without compromising the mechanical stability of the structure as well as their fibrillar structure. Thus, by controlling the granule size and their concentration, it is possible to control the pore size and, consequently, the cellular infiltration within fibrous mats. This study demonstrated the ability of either glucose or CS grains to be used with electrospun mats to improve cell infiltration.

Keywords
Electrospinning; Chitosan grains; Glucose grains

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Abstract **#1988**

NOTCH SIGNALING REGULATES STRAIN-MEDIATED CHANGES IN VASCULAR SMOOTH MUSCLE CELL PHENOTYPE

Cansu Karakaya$_P$[1,2], Valery Visser[1], Tommaso Ristori[1,2,3], Carlijn Bouten[1,2], Cecilia Sahlgren[1,2,4], Sandra Loerakker[1,2]

[1] *Department of Biomedical Engineering, Eindhoven University of Technology*

[2] *Institute for Complex Molecular Systems, Eindhoven University of Technology*

[3] *Department of Biomedical Engineering, Boston University*

[4] *Faculty of Science and Engineering, Åbo Akademi University*

Corresponding author's email: c.karakaya@tue.nl

In situ vascular tissue engineering aims at regenerating vascular tissues in terms of form and function. Currently, some fundamental questions, e.g. how engineered tissues remodel after implantation and how a native-like organization can be achieved to enable proper functionality, remain to be answered. To address these challenges, a mechanistic understanding of the processes mediating functional vessel growth and remodeling in engineered tissues is needed, particularly concerning the interplay between mechanical cues and cell-cell signaling. Vascular smooth muscle cells (VSMCs) can switch their quiescent contractile phenotype to a synthetic phenotype for growth and remodeling. This switch is regulated by mechanical cues [1] and Notch signaling [2] which is mechanosensitive [3,4]. A computational model has predicted that the mechanosensitivity of Notch signaling leads to a phenotypic switch in VSMCs to establish homeostasis [4]. Yet, these computational findings are scarcely validated, and the interaction between mechanical cues and Notch signaling in the remodeling of engineered vascular tissues is poorly understood. In this study, control and Notch-inhibited synthetic and contractile VSMCs were equibiaxially stretched for 48 hours. IF imaging and qPCR were conducted to characterize the changes in cell phenotype, ECM production and Notch signaling activity. Our results showed that Notch signaling needs to be active for the maintenance of contractile phenotype. The application of strain resulted in a transition in contractile VSMCs towards the synthetic phenotype with a decreased Jagged1 expression. The current study suggests that Notch signaling has a key role in regulating strain-mediated changes in VSMC phenotype.

Keywords

mechanoregulation; cell-cell signaling; remodelling of engineered tissues

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Abstract **#1989**

3D PRINTED BIOACTIVE COMPOSITE SCAFFOLDS FOR TREATING LARGE OSTEOCHONDRAL DEFECTS IN PORCINE MODEL

Sumit Murab[P][1,2], Jenna Hall[2], Stacey Gruber[3], Chia-Ying James Lin[2,3], Patrick Whitlock[1,2,3]

[1] *Division of Pediatric Orthopaedic Surgery, Cincinnati Children's Hospital Medical Center*

[2] *Division of Orthopaedic Surgery, University of Cincinnati College of Medicine*

[3] *Department of Biomedical Engineering, University of Cincinnati*

Corresponding author's email: sumit.murab@cchmc.org

Existing clinical treatments of large osteochondral defects often use autografts and allografts as “gold standards”. Limitations include donor site morbidity and scarcity for large defects, risk of transfection and difficulty treating complex geometries. Implanted allografts possess reduced mechanical and biologic properties when compared with native tissue (1). Similarly, tissue engineered constructs are often produced using gels or polymers that are often too weak to withstand in vivo forces experienced by osteochondral tissue (2). We previously demonstrated that decellularized cartilage/ bone extracellular matrix (DCM) within a thermally protective polylactic acid (PLA) microsphere barrier can be 3D-printed within polycaprolactone (PCL) filament, maintaining the bioactivity of the DCM (3). In this study, a 6 mm osteochondral defect was treated in a Yucatan mini-pig model (protocol # IACUC2020-0081) with a biphasic, 3D-printed PCL scaffolds containing human bone and cartilage DCM encapsulated in PLA microspheres. Scaffolds with blank PLA microspheres and autografts were used as controls. After 3 months, preliminary histological results (H&E, Toluidine blue & Masson’s Trichrome) demonstrated formation of smooth, cartilage in the articular phase that appeared to integrate well with the underlying subchondral bone phase in the biphasic DCM scaffolds. The deeper osteo-conducive phase displayed evidence of bone tissue formation around the scaffold struts. The blank scaffolds showed formation of fibrocartilage tissue on the articulating surface with bone regeneration around the scaffold. The autografts showed cartilage with decreased GAG content in toluidine blue staining. Overall, the results demonstrated enhanced osteochondral regeneration in biphasic DCM scaffolds comparable with controls.

Keywords

osteochondral; 3D printing; decellularized matrix

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Abstract **#1990**

EVALUATION OF A 3D PRINTED CELLULAR MATRIX FOR CARTILAGE DEFECT REPAIR IN A CANINE MODEL

Charles R. Bragdon[P][1,2], Yhan Colon Iban[2], Jina Ryu[3], Eiji Kenazawa[2], Martin Magneli[2], Minju Kim[3], 3.Jiyeon Moon[3]

[1] *Harvard University*

[2] *Massachusetts General Hospital*

[3] *Rokit Healthcare*

Corresponding author's email: Jay.gu@rokit.co.kr

Introduction: The ability to repair damaged hyaline cartilage could revolutionize the treatment of sports injuries and end osteoarthritis. Methods: A canine bilateral medial femoral condyle cartilage defect model, using 12 adult male beagles, was used to evaluate the effectiveness of a 3D printed cellular matrix composed of fibrin glue, lyophilized human chondral cartilage (LCCM), and stem cells harvested from patients undergoing abdominal liposuction, for treating articular cartilage defects. Results: In contrast to the control groups, the defects treated with fibrin glue, LCCM and stem cells had striking visual, histological, immunochemistry and mechanical similarities to that of normal articular cartilage. The tissue in these defects appear to be developing centrally out to the periphery as well as to be healing from the periphery inward. At both 20 and 32 weeks much of the defect appears occupied by new thick white cartilage-like tissue. In addition, there appears to be lamellar structures within this tissue which blends with the existing normal cartilage. The mechanical stiffness of the new tissue was similar to that of normal cartilage. Upon microscopic examination, there was a well-defined surface layer covering the middle layer of chondrocytes which were surrounded by a matrix composed of Collagen I, Collagen II, and proteoglycans, all similar in appearance to the normal cartilage control. Discussion: The results of this study represent one of the best examples of articular cartilage healing in an animal model. A clinical trial is now underway using this new technology.

Keywords

Hyaline Cartilage Repair; 3-D Matrix; Chondrocytes

Abstract **#1991**

DEVELOPMENT AND CHARACTERIZATION OF BIODEGRADABLE COLLAGEN SCAFFOLDS FUNCTIONALIZED WITH SDF-1A LOADED HEPARIN-BASED HYDROGEL AND POLYHEXAMETHYLENE GUANIDINE HYDROCHLORIDE LOADED ALGINATE MICROSPHERES

Ianina Pokholenko$_P$[1], Olena Toporova[1], Svitlana Rymar[1], Olena Moshynets[1], Polina Pikus[1], Vitalii Kordium[1]

[1] *Institute of Molecular Biology and Genetics of NASU*

Corresponding author's email: yasnenka@gmail.com

Aim: Present study focuses on the development and characterization of biodegradable collagen scaffolds functionalized with SDF-1a loaded heparin-based hydrogel and polyhexamethylene guanidine hydrochloride (PHMG) loaded alginate microspheres for promoting in vivo tissue regeneration.

Methods: The scaffolds were prepared by freeze-drying of bovine atelocollagen I solution containing polymer based on cross-linked modified heparin or PHMG loaded alginate microspheres. Bone marrow MSC were isolated from FVB-CgTg(GFPU) 5Nagy/J mice. Distribution of the cells within collagen scaffolds was studied by CLSM. The recruitment of MSCs was studied in allogenic transplantation model. The anti-microbial activity of developed PHMG loaded scaffolds was evaluated by agar diffusion method.

Results: The developed collagen scaffolds functionalized with heparin-based hydrogel can be efficiently loaded by recombinant SDF-1a. The maximum binding capacity observed was 14.83 ± 3.4 μg SDF-1a per 1 mg of hydrogel. It has been shown that developed hydrogel was able to provide gradual release of the protein within 72 hours in vitro. The developed scaffold with PHMG loaded alginate microspheres reduced microbial growth of gram-negative bacteria. Functionalization of collagen scaffold by SDF-1a loaded heparin-based hydrogel increases the seeding efficiency of MSC and promotes the colonization and remodeling of the scaffold both in vitro and in vivo.

Conclusions: The developed biodegradable collagen scaffolds functionalized with SDF-1a loaded hydrogel and PHMG loaded alginate microspheres can accelerate wound healing activity by prevention of bacterial infection and stimulation of host MSC recruitment.

Abstract **#1992**

EVALUATION OF IMMUNOMODULATORY MESH FOR PELVIC FLOOR RECONSTRUCTION IN A RABBIT COLPOPEXY MODEL

Bryan Brown$_P$[1], Aimon Iftikhar[1], Alexis Nolfi[1], Clint Skillen[1], Branimir Popovic[1], Meegan Ambrose[1], Pamela Moalli[1]

[1] *University of Pittsburgh*

Corresponding author's email: brownb@upmc.edu

The use of mesh in pelvic organ prolapse repair has improved outcomes associated with native tissue repairs. However, a significant number of women with mesh experience complications, often requiring surgical revision. This study demonstrates a method for modulating the early host macrophage response with interleukin-4 (IL-4) coated mesh to lessen the pro-inflammatory response and promote tissue integration. Studies were performed in an adapted lumbar colpopexy in rabbits. The host response and tissue remodeling outcomes were evaluated at 14 days and late stages 90 days. In vitro testing demonstrated that the IL-4 coating was bioactive following terminal sterilization of the coated mesh, promoting macrophage transition to an anti-inflammatory phenotype. When implanted, IL-4 coated mesh materials were shown to shift the host macrophage response towards a more anti-inflammatory, M2 macrophage phenotype at 14 days as compared to uncoated polypropylene mesh. This shift resulted in improved mesh integration, decreased tissue degradation, improved tissue mechanical strength, and a reduction in intraabdominal adhesions representing significant improvements in the remodeling outcome. These data demonstrate that modulation of the early host response to polypropylene mesh toward an M2 phenotype has the potential to result in significant improvements in mesh integration, reduction in tissue degradation and associated complications, and overall clinical success.

Abstract **#1993**

PHYSICAL DECELLULARIZATION USING CYCLIC APPLICATION OF HIGH HYDROSTATIC PRESSURE

Shogo Miyata[P][1], Masashi Yamamoto[2], Daiki Zemmyo[2]

[1] *Department of Mechanical Engineering, Faculty of Science and Technology, Keio University*

[2] *Graduate School of Science and Technology, Keio University*

Corresponding author's email: miyata@mech.keio.ac.jp

Decellularized tissues are promising materials that mainly consist of extracellular matrices generated by removing cells from biological tissues. As for physical decellularization method, high hydrostatic pressure (HHP) has been used to remove cells physically from organs or tissues rather than by biochemical reagents. However, ultrahigh hydrostatic pressure induces denaturation of the ECM structure. In this study, the effects of cyclic HHP on the structure of cell were examined to establish a novel decellularization method without the denaturation of the ECM. A decellularization device that enabled to impose cyclic HHP was developed. Human skin cell suspension was injected into a plastic bag to be subjected to cyclic HHP. After applying cyclic HHP, the amount of DNA and the morphological changes of the cells were evaluated. As a result, the amount of DNA decreased by the cyclic HHP application compared to the static HHP. Moreover, cyclic HHP was suggested to induce the denaturation of the structure of nuclear membrane. In summary, it was revealed that the cell structure could be denatured and destroyed by cyclic HHP application at a lower level than that of previous studies.

Keywords

High hydrostatic pressure; Cyclic pressure; Decellularization

Abstract **#1994**

GLYCOSAMINOGLYCAN-BASED SMART HYDROGELS FOR EX VIVO FORMATION OF SCAFFOLD-FREE VASCULAR CAPILLARIES

Mikhail V. Tsurkan[P][1,2], Simone Arndt[2], Sarah D. Tsurkan[1,2]

[1] *Leibniz Institute for Polymer Research Dresden e. V. (IPF)*

[2] *TissueGUARD GmbH*

Corresponding author's email: tsurkan@ipfdd.de

Angiogenesis, the outgrowth of blood vessels, is crucial in the stabilization of implanted tissue and regeneration of various clinical pathologies. Creating implantable blood vessels in vitro remains challenging because the capillary morphogenesis of endothelial cells (ECs) is controlled by multiple exogenous signals. Moreover, for clinical applications, such development requires a fully synthetic material with known concentration and bioavailability of each component. Recent advances in tissue engineering allowed for creating and maintaining stable vascular capillaries over periods of about one month in vitro in situ-forming matrix metalloproteinase-degradable starPEG-heparin hydrogels containing covalently bound integrin ligands and reversibly conjugated pro-angiogenic growth factors (SDF, bFGF, VEGF) [1]. But currently used technologies did not allow for the entire release of produced blood vessels from their culture platform. Our lab has established a technology that enables us to transfer any peptide crosslinked materials into a trigger responsive smart scaffold by including a thrombin cleavable five amino acid sequence into the peptides. In such material, the matrix metalloproteinase-degradable sequence allowed for the cell expansion and the formation of the blood vessels, and when the tissue is ready, the addition of the thrombin biorthogonality removes the scaffold allowing of the harvesting of the undamaged vessels. In a model experiment, we have shown how such smart material can be used to form, harvest, and transplant a vial capillary network. Although we focus on the blood vessels in our work, this“smart” material design can be utilized for tissue or organoids formation, gentle harvest, and transplantation with corresponding adjustments.

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Chwalek K, Tsurkan MV, Freudenberg U, Werner C. Scientific Reports 4 2014. 4414

Abstract **#1995**

MANNOSE-DECORATED SUPRAMOLECULAR ASSEMBLIES TO TARGET THE MANNOSE RECEPTOR ON MACROPHAGES AND KIDNEY CELLS

Laura Rijnsp[1,2,3], Lu Su[4,3], Yan Ni[2,3], Bert Meijer[4,3], Patricia Dankers[1,2,3]

[1] *Department of Biomedical Engineering, Laboratory for Cell and Tissue Engineering, Eindhoven University of Technology*

[2] *Department of Biomedical Engineering, Laboratory of Chemical Biology, Eindhoven University of Technology*

[3] *Institute for Complex Molecular Systems, Eindhoven University of Technology*

[4] *Department of Chemical Engineering, Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology*

Corresponding author's email: l.rijns@tue.nl

The mannose receptor (CD206) is a transmembrane protein expressed on most macrophages and kidney mesangial cells.1,2 CD206 is involved in many immunological processes; CD206 activation leads to the release of a variety of cytokines, like interleukin-6 and -10.3

To better understand the binding mechanisms underlying mannose receptor activation, we here propose synthetic supramolecular glycopolymers to target CD206 in both RAW 264.7 macrophages and human renal mesangial cells (hRMCs). These precisely-defined and tunable supramolecular assemblies allow us to assess the effect of a single microenvironment property (e.g. mannose concentration) on mannose receptor behavior. For the supramolecular glycopolymers, we propose several different mannose-decorated benzene-1,3,5-tricarboxamides (BTAs),4 which can self-assemble into fiber-like structures through a combination of hydrogen bonding, π- π interactions and the hydrophobic effect5,6. Here, we investigate the effect of multivalency (1, 2 or 3 mannose units) and secondly, we test the influence of accessibility (with or without ethylene glycol linker) on the binding affinity and cellular behavior.

It was demonstrated that CD206 is present on hRMCs in low amounts (~ 17%) and that ~ 50% of RAW 264.7 macrophages are CD206+. We then showed that the mannose BTA assemblies do not exhibit toxic effects towards hRMCs at 100 µM concentrations. Lastly, we observed a higher binding affinity towards CD206 for mannose-3 BTA (Kd ~ 20-50 µM) than for mannose-2 BTA (Kd of 200 µM), illustrating the multivalency effect. Next, we will assess the cellular effects of CD206 targeting. Eventually, our results will lead to better understanding of the cell-biomaterial interaction.

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Abstract **#1997**

EXTRACELLULAR MATRIX FUNCTIONALIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-BASED CARDIAC TISSUES IMPROVES CARDIOMYOCYTE MATURATION

Henrique V. Almeida[1,2], Miguel Tenreiro[1,2], Ana F. Louro[1,2], Bernardo Abecasis[1,2], Deolinda Santinha[3,4], Tomás Calmeiro[5], Elvira Fortunato[5], Lino Ferreira[3,4], Paula M. Alves[1,2], Margarida Serra[1,2]

[1] IBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal

[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

[3] CNC, Centro de Neurociências e Biologia Celular, Universidade de Coimbra, 3004-517 Coimbra, Portugal

[4] Faculdade de Medicina, Universidade de Coimbra, Rua Larga, 3004-504 Coimbra

[5] CENIMAT|i3N, Departamento de Ciência dos Materiais, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

Corresponding author's email: henrique.almeida@ibet.pt

Human induced pluripotent stem cells (hiPSC) have a great potential in the development of novel cardiac therapies due to their high self-renewal capability and potential to differentiate into cardiomyocytes (CM). However, generated hiPSC-derived CM (hiPSC-CM) are still immature, and this has been limiting their application. Recent findings have demonstrated extracellular matrix (ECM) potential as a key regulator in the development, homeostasis, and injury of the in vivo cardiac microenvironment [1]. This work's objective was to assess the impact of human cardiac ECM in the maturation of hiPSC-CM. Human ECM was isolated from the myocardium of three healthy donors through physical decellularization. The ECM was cryomilled and characterized in terms of particle size and composition. These ECM particles were then incorporated in a 3D model of hiPSC-CM [2], cultured as aggregates in dynamic stirred culture. The hiPSC-CM were characterized after two weeks of culture.

The results showed that the cardiac tissue decellularization process reduced DNA content (>75%) and maintained ECM composition. The cardiac ECM particles were successfully incorporated in 3D hiPSC-CM aggregates (ECM+hiPSC-CM), with no impact on cell morphology, aggregate size, composition, and metabolic activity. The ECM+hiPSC-CM aggregates showed beating rates closer to physiologic values, more organized and longer sarcomeres, and improved calcium handling than standard hiPSC-CM aggregates.

This work shows for the first time that human cardiac ECM promotes the structural and functional maturation of hiPSC-CM. The knowledge generated provides important insights to strengthen the application of hiPSC-CM in cell therapy, drug discovery, and cardiac disease modeling.

Keywords

Extracellular Matrix; Cardiomyocyte Maturation; 3D Culture

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Abstract **#1998**

ADIPOSE-DERIVED STEM CELLS AS A PROMISING MODEL FOR STUDYING ACTIVITY OF PEPTIDES FOR CARTILAGE AND BONE REGENERATION.

Michal Pikula[P][1], Milena Deptula[1], Martyna Fularczyk[2], Maria Dzierzynska[2], Patrycja Gzowska[1], Agata Tyminska[1], Aneta Skoniecka[1], Jacek Zielinski[3], Sylwia Rodziewicz-Motowidlo[2]

[1] *Department of Embryology, Medical University of Gdansk, Poland*

[2] *Department of Biomedical Chemistry, University of Gdansk, Poland*

[3] *Department of Surgical Oncology, Medical University of Gdansk, Poland*

Corresponding author's email: PIKULA@GUMED.EDU.PL

Adipose-derived stem cells (ASCs) have been the subject of intensive basic research and clinical trials. These cells show many biological activities related to the secretion of multiple cytokines or growth factors, and immuno-regulatory effects. In clinical trials, ASCs are used in the treatment of many diseases, including tissue reconstruction and chronic wounds. ASCs may also serve as an in vitro model for studying the activity of drug candidates. In vitro, ASCs can be differentiated into adipocytes, chondrocytes and osteocytes, therefore, they are a good model for studying differentiation stimulants. In our study, we have shown that stem cells isolated from fat tissue (constituting medical waste after oncological surgery procedures) can be successfully used in the above-mentioned model. Bone morphogenetic proteins (BMPs) play an important role in the proliferation and differentiation of a many cells, especially bone and cartilage cells. BMPs also stimulate differentiation of mesenchymal stem cells into osteocytes and chondrocytes. In our research, we used chemically synthesized peptides with structure based on the human BMPs protein. Some of them in vitro stimulated proliferation, migration of ASCs and collagen synthesis. Their effect on ASCs differentiation was also evaluated. These peptides could potentially be used for in vitro stimulation of bone and cartilage formation for therapeutic purposes. Furthermore, they could be combined with scaffolds for transplantation in different orthopedic conditions related to bones and cartilages damage (fracture, loss, injury, inflammation).

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Keywords

Adipose-derived stem cells ; Regeneration; Peptides

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Abstract **#1999**

HYBRID LAMININ-BASED HYDROGELS FOR EFFICIENT PRESENTATION OF GROWTH

Oana Dobre[P][1], Sara Trujilio[1], Matthew J. Dalby[1], Manuel Salmeron-Sanchez[1]
[1] *Centre for the Cellular Microenvironment, University of Glasgow.*
Corresponding author's email: oana.dobre@glasgow.ac.uk

Introduction: Hydrogel systems can be engineered with the aim of regenerating different tissues (e.g. bone and vascular). Protein-based hydrogels are appealing for their structural designability, specific biological functionality, and stimuli-responsiveness [1][2][3]. Here, we present 3D Poly (ethylene glycol)-laminin (PEG-LM) hydrogels for delivery of growth factors in a controlled manner.

Methods: 3D laminin-based hydrogels containing different concentrations of PEGylated human laminin with acrylates were crosslinked via photopolymerisation with two- or four-arm acrylate and a protease-degradable peptide (VPM). Human mesenchymal stem cells from bone marrow (hMSCs) and human umbilical vein endothelial cells (HUVECs) with different growth factors were incorporated into the laminin hybrid hydrogels to evaluate cell cytotoxicity, study the controlled release of the growth factors and their phenotypical potential to driven different cell linages.

Results: We studied the ability of the hybrid PEG-laminin hydrogels to promote regeneration of different tissues (e.g. bone and vascular) by delivering growth factors in a controlled manner. Immunofluorescence and qRT-PCR results of hMSC encapsulated in 3D LM/PEG hydrogels loaded with BMP-2 showed an upregulation of osteopontin expression at 14 days. More, LM/PEG hydrogels loaded with vascular endothelial growth factor (VEGF) showed a greater potential to promote endothelial cell sprouting after 4 days in culture.

Conclusion: We report on the development and characterization of hybrid 3D laminin hydrogels with tuneable mechanical and degradable properties and highly efficient growth factor presentation for potential tissue regeneration of bone and vascular tissues.

Acknowledgements: This work was completed with support from the Engineering and Physical Sciences Research Council (EPSRC) Grant EP/P001114/1.

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Abstract **#2000**

ELECTRICALLY AND THERMALLY STIMULATED HYDROGEL BASED ON CARBON NANOTUBE SPONGE FOR SWITCHABLE DRUG DELIVERY

Sang-Yu Park[P][1,2], Ueon Sang Shin[2], Ji-Young Hwang[1]

[1] *Korea Institute of Carbon Convergence Technology*

[2] *Dankook University*

Corresponding author's email: jyhwang@kctech.re.kr

Hydrogels as one of smart materials that are controllably responsive to external (e.g. electrical or thermal) stimulation have become increasingly popular in recent years for simple, rapid and precise drug delivery that can be controlled by simply switching the external stimulus on or off. However, achieving highly uniform distribution of the hydrophobic electrically conductive materials throughout the hydrophilic three-dimensional (3D) hydrogel network remains a challenge, and is essential in achieving a well-connected electrical and thermal conducting path. This study investigated electrical and thermal-responsive hydrogels based on carbon nanotubes (CNTs) as the electrical/thermal conductor and core unit, chitosan (Chit) as a hydrophilic dispersant and shell unit, and poly(NIPAAm-co-BBVIm) (pNIBBIm) as a temperature-responsive copolymer and drug carrier. Uniform distribution and 3D connectivity of CNTs was improved by formulating the CNT-core and Chit-shell units and constructing a CNT sponge framework. The hydrogel based in the CNT sponge, namely the 3D framed CNT-Chit/pNIBBIm hydrogel, exhibited the best potential for future use in a drug delivery system. The physiochemical, mechanical, electrical, thermal, and biocompatibility characteristics of the 3D framed hydrogel led to remarkable electrical- and thermal-responsive properties that allowed for the development of an excellent controllable and switchable drug delivery platform for tissue engineering and regeneration applications.

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Abstract **#2001**

MICROENVIRONMENTAL ARRAYS AND COMPUTATIONAL MODELLING TO DELINEATE CO-OPERATIVE EFFECTS OF NOTCH SIGNALING AND BIOMECHANICAL CUES IN PATTERNED LIVER DIFFERENTIATION

Ishita Jain[P][1], Gregory Underhill[1]

[1] *University of Illinois at Urbana Champaign*

Corresponding author's email: ij3@illinois.edu

The bipotential liver progenitors can differentiate into both hepatocytes and biliary epithelial cell fates. Previously, it has been shown that when these bipotential liver progenitor cells are cultured on a defined circular geometry, a patterned differentiation is observed. The peripheral cells differentiate into biliary epithelial cells, while the interior cells differentiate into hepatocytes. It was found that the cells on the periphery were more contractile and notch signaling was essential for the pattern. Since, notch has been implicated in various diseases such as the Alagille Syndrome, we perturb expressions of notch ligands DLL1 and Jagged-1 to further deduce their exact roles in combination with the cell-substrate interactions, in the differentiation pathway. Biliary differentiation on the circular islands significantly increased with DLL1 knockout (KO) cells whereas it diminished on Jag1 KO cells. A computational model was also developed by simulating the concentration of Notch receptor, active Notch intracellular domain and the 2 notch ligands in each cell. In addition to that, we incorporated cell-cell interaction and cell traction forces in the model with constant feedback between the neighbor cells. Overall, we found that Jagged-1 promotes biliary differentiation, while DLL1 limits it to the periphery. Secondly, we saw more biliary differentiation on the stiffer substrate when DLL1 is knocked out. Lastly, upon perturbation of the various ligand concentrations and traction force profiles in a computational model for the cell differentiation, we emulate our experimental results. Hence, our experimental observations and the computational model reports novel insights in the liver differentiation pathway.

Abstract **#2002**

DISSECTING THE MICROENVIRONMENTAL CONTROL OF LIVER STELLATE CELL EPIGENETICS AND FIBROGENIC PHENOTYPES

Ishita Jain[P1], Aidan Brougham-Cook[1], Gregory Underhill[1]

[1] *University of Illinois at Urbana Champaign*

Corresponding author's email: ij3@illinois.edu

Non-alcoholic steatohepatitis (NASH) is an emerging epidemic of liver disease and the basis for a rising incidence of fibrosis and hepatocellular carcinoma. Hepatic stellate cells (HSCs) have been identified as the primary drivers of liver fibrosis. Changes in the composition of the liver microenvironment during fibrosis results in a complex crosstalk of extracellular cues that promotes the activation, or differentiation, of HSCs from a quiescent, vitamin A storing phenotype to a myofibroblastic phenotype. Previously, we analyzed and found distinct phenotypic behaviors of HSCs in our high throughput analysis of various ECM combinations and substrate stiffness. Here, we further wanted to delineate the key mechanisms for those behaviors, namely the epigenetic state. Epigenetic mechanisms have been implicated in the activated phenotype of HSCs, however how they change with the dynamic microenvironment has not been quantified. we delineated the various epigenetic states of HSC's, specifically for those microenvironments that have shown 3 distinct phenotypic behaviors. This resulted in distinct epigenetic clusters, where the cells on the soft substrates had the most epigenetic activity. Lastly, we quantified nuclear shape parameters which have shown to guide chromatin function. One such parameter was nuclear volume, which was significantly higher in cell cultured on 1 kPa, signifying higher gene accessibility. Ongoing efforts are focused on getting a genome-wide epigenetic sequencing to figure out the key genes regulated by these markers.

Abstract **#2003**

UNCOUPLING MECHANICAL STRETCH FROM CONTRACTILE FUNCTION OF ENGINEERED CARDIAC TISSUES TO BETTER MIMIC CARDIAC CYCLES

Gaetano Scuderi[1], Anuush Vejalla[1], Andrea Lucia Alfonso[1], Brianna Hou[1], Daniel Cheung[1], Terence Gee[1], Vincent Zhang[1], Kathleen Clifford[1], Jonathan Butcher[1]

[1] *Cornell University*

Corresponding author's email: Gjs95@cornell.edu

Current mechanical stimulation bioreactor systems of engineered cardiac tissues (ECTs) utilize stiff anchorage points to stretch the tissues. Therefore, the ECTs in these systems can only undergo isometric contractions and are unable to produce work, which hinders their maturation and can lead to an upregulation of pathological fibrosis. Thus, we developed a high-throughput mechanical stimulation bioreactor system that uncouples mechanical stretch from the contractile function of uniaxially aligned 5mm long ECTs suspended on one flexible polydimethylsiloxane post and one immobile polydimethylsiloxane post. This engineered bioreactor system uniaxially stimulates 48 ECTs simultaneously from 0-25% strain and 1-5 Hz by deflecting the flexible post above the ECTs utilizing a stiff tab grid system that is not directly connected to the anchored ECTs. ECTs composed of embryonic chick cardiac cells were cultured statically for 7 days followed by two different stimulation regimens for an additional 7 days: 1) static (non-stretched) and 2) uniaxial constant cyclic stretch of 12.5% strain and 1 Hz frequency. The cyclically stretched ECTs and static ECTS maintained an average spontaneous beating frequency of 1.06 Hz and 0.27 Hz respectively and average resting lengths of 5mm and 3.2mm respectively. However, there was no statistically significant difference in overall spontaneous contractile force between stimulation groups. These results suggest constant mechanical stretch affects contractile frequency and the maintenance of resting length but not necessarily the force of spontaneous contractions. Future work aims to include duty cycles and increasing frequency regimens to study how varying mechanical stimulation parameters affect ECT functionality.

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Abstract **#2004**

INFLUENCE OF MECHANICAL AND TGFB3 STIMULATION ON TENOGENIC DIFFERENTIATION OF TONSIL-DERIVED MESENCHYMAL STEM CELLS

Hyang Kim[P][1], Junghwa Choi[1], Saerom Jeong[2], Mi Na Choi[1], Sang-Jin Shin[2], Seung Yeol Lee[1,3]

[1] *New Horizon Biomedical Engineering Institute, Myongji Hospital*

[2] *College of Medicine, Ewha Womans University*

[3] *College of Medicine, Hanyang University*

Corresponding author's email: kernels00@naver.com

The purpose of this study was to investigate the effects of mechanical strains and TGF-β3 on tenogenic differentiation of tonsil-derived mesenchymal stem cells (TMSCs) and to evaluate the expression of tendon-related genes and ECM components such as collagen. The tonsil-derived mesenchymal cells (TMSCs) were subjected to two types of treatment: TGF-β3 and mechanical strain for 1, 3, and 7 days. Under each condition, the mRNA expression level was measured by qRT-PCR and dsDNA was measured to assess cell proliferation and normalize collagen content as an end product. Collagen concentration was assessed using the Sircol Collagen Assay Kit. The mRNA expression of tenogenic genes such as SCX, Decorin, COL1, and COL3 was significantly higher when mechanical strain was applied than under the static condition. However, the mRNA expression of tenogenic genes was not significantly different according to the intensity of mechanical strain with TGF-β3. In cells without TGF-β3 treatment, dsDNA concentration decreased, while the amount of normalized collagen increased as the intensity of mechanical strain rose. Alternatively, in cells treated with TGF-β3, the collagen concentration did not change according to the intensity of the mechanical strain, while dsDNA concentration decreased when mechanical strain was applied. The mRNA expression of osteogenic and chondrogenic genes was not significantly different among the static, 2%, and 5% mechanical strain, regardless of TGF-β3 treatment. In conclusion, mechanical strain and TGF-β3 have significant effects on TMSC differentiation into tenocytes. However, a combination of the two does not have a synergistic effect on differentiation.

Keywords

Tonsil-derived mesenchymal cells; TGF-β3; Tenogenesis

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Abstract **#2005**

HARNESSING IMMUNE CELL PSEUDOTIME TRAJECTORY ANALYSIS TO INFORM REGENERATIVE MEDICINE STRATEGIES IN CRITICALLY SIZED VOLUMETRIC MUSCLE LOSS INJURY

Lauren A Hymel[P1], Shannon E Anderson[1], Molly E Ogle[1], Thomas C Turner[1], William Y York[1], Hong Seo Lim[1], Young C Jang[2,3], Nick J Willett[2,4,5], Edward A Botchwey[1,2]

[1] *Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332*

[2] *Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332*

[3] *School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332*

[4] *Department of Orthopedics, Emory University, Atlanta, GA 30322*

[5] *Atlanta Veteran's Affairs Medical Center, Decatur, GA 30030*

Corresponding author's email: lhymel3@gatech.edu

While skeletal muscle has remarkable capacity for repair following acute injuries, volumetric muscle loss (VML) injuries overwhelm endogenous repair mechanisms, leading to failed regeneration, fibrosis, and loss of function [1, 2]. Following injury, immune cell trafficking within the injury milieu is tightly coordinated with muscle regeneration [3]; thus, its regulation is crucial. We characterized the local infiltration and phenotypic complexity of immune cells responding to critically sized VML defects versus subcritical defects in a pre-clinical model to elucidate the cellular players underlying a pathological or functional outcome. We employed dimensionality reduction and pseudotime analysis on single-cell flow cytometry data from injured muscle to reconstruct cellular transitions and reveal heterogeneous subsets of immune populations plaguing critical injuries. We found significantly increased M2 macrophages co-expressing TNFα and TGFβ persisting within critical VML defects compared to subcritical defects, indicative of perturbed M1-to-M2 macrophage transitions which may induce fibrosis and impair regeneration [4]. Production of sphingosine-1-phosphate (S1P) and differential expression of its 5 receptors (S1PR1-5) within macrophages have potent effects on mediating a pro-reparative microenvironment [5]. We show that local delivery of VPC01091 (S1PR3 antagonist) from nanofibers to critical defects results in a reduction of inflammatory relative to anti-inflammatory macrophages, an expanded muscle stem cell pool, and increased bridging of regenerating myofibers across the defect. Not only do these findings reveal how unique immune cell phenotypes may contribute to VML pathology but further indicate how S1P receptor modulators can be re-purposed to locally target endogenous repair cells as a novel form of regenerative immunotherapy.

Keywords

Inflammation; Immunomodulation; Regeneration

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Abstract **#2006**

RESOLUTION-BASED IMMUNOMODULATORY PEG-MAL HYDROGELS ACHIEVE PRO-REGENERATIVE IMMUNOMODULATION AFTER CRITICAL VOLUMETRIC MUSCLE LOSS INJURY

Thomas Turner$_P$[1], Lauren Hymel[1], Mary Caitlin Sok[1], Edward Botchwey[1]

[1] *Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA*

Corresponding author's email: tturner42@gatech.edu

Severe skeletal muscle trauma resulting from volumetric defects is an intractable problem involving the irrecoverable loss of tissue that results in dysfunction and disability [1]. Thus, identifying effective interventions to improve muscle regeneration following such an injury or disuse has immediate clinical value. The mechanistic link between muscle regeneration and the inflammatory cascade has been shown to be vital in the functional recovery of muscle tissue [2]. Specialized pro-resolving lipid mediators' (SPM) active role in the resolution of inflammation has opened up an exciting new avenue of a resolution-based approach to immunomodulatory regenerative medicine strategies [3]. However, the field of resolution pharmacology has only begun to consider the possibility of engineering tools for local and more highly successful SPM delivery. Previously, we have investigated the advantages the biomaterial-based release of SPMs, specifically Aspirin-Triggered Resolvin D1 (AT-RvD1), has in manipulating and directing the recruitment of pro-regenerative immune cell subsets in vivo for improved tissue regeneration [4]. Here, using LC-MS/MS analysis, we discover a dysregulated SPM metabolism after a critical volumetric muscle loss defect. Thus, we develop a PEG-MAL hydrogel platform for the delivery of AT-RvD1, and its SPM precursors, to a critical volumetric muscle loss (VML) injury model in the murine quadricep. We leverage novel dimensionality reduction and pseudotime analytical techniques to characterize the host immune response to this immunomodulatory hydrogel via in vivo high-dimensional single-cell flow cytometry. Notably, we observe enhanced inflammation resolution characterized by increased accumulation of anti-inflammatory monocytes and a regenerative phenotype of macrophages, while limiting neutrophil infiltration.

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Abstract **#2007**

A CELLULAR- AND BIOMATERIAL- TOOLBOX FOR THE DEVELOPMENT OF A 3D IN VITRO ENGINEERED BONE FRACTURE CALLUS

Johanna Bolander[P][1,2], Carlos Mota[3], Huey Wen Ooi[3], Hannah Agten[1,2], Matthew B Baker[4], Lorenzo Moroni[4], Frank P Luyten[1,2]

[1] Tissue Engineering Laboratory, Skeletal Biology and Engineering Research Center, KU Leuven, Belgium.

[2] Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Belgium.

[3] Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands.

[4] Matthew B. Baker3

Corresponding author's email: jbolande@wakehealth.edu

In the development of a cell-based construct, progenitor cells are crucial to integrate and form tissues with available host cells, stimulatory factors to direct cellular processes and biomaterials to provide cells with 3D cues. We aimed to develop a cell-based construct designed to mimic the endochondral fracture callus based on human periosteum derived cells (hPDCs), combined with a unique norbornene functionalized alginate system as a bioink. For the final assembly of the engineered callus, bioprinting was used to allow precise and controlled 3D deposition.

Preconditioned cells were seeded for condensation in a high throughput aggregation system and primed under normoxic or hypoxic conditions for 6 days followed by in vitro analysis and in vivo assessment. Synthetic biocompatible bioinks were developed based on a norbornene functionalized alginate system, resembling the specific extracellular matrix (ECM) stiffness present in the different zones of the endochondral callus. Based on statistical scoring, the cellular profiles in regard of cellular metabolism, marker genes and matrix formation, culture regimens that promoted early chondrogenic, late chondrogenic and osteogenic differentiation could be defined. When these cell populations were encapsulated in the biocompatible bioinks, tissue development was further supported. The final constructs were assembled through bioprinting in a spatially organized tissue followed by ectopic assessment, which led to further in vivo tissue development and maturation.

We herein report a bioinspired method for the in vitro engineering of a long bone fracture callus where step-wise in vitro priming prepares and steer cell differentiation, further supported in vivo by the framing biomaterials.

Keywords

Fracture callus; Critical size bone defects; Periosteum

Abstract **#2008**

AFFINITY-CONTROLLED DELIVERY OF A THERMO-STABILIZED VARIANT OF CHONDROITINASE ABC AS A THERAPEUTIC STRATEGY FOR STROKE

Nitzan Letko Khait[P][1], Sabrina Zuccaro[1], Molly Shoichet[1,2,3]

[1] *Department of Chemical Engineering and Applied Chemistry, University of Toronto*

[2] *Institute of Biomedical Engineering, University of Toronto*

[3] *Department of Chemistry, University of Toronto*

Corresponding author's email: nitzan.letko@utoronto.ca

Injuries to the central nervous system (CNS), including spinal cord injury and stroke, are characterized by the formation of a proteoglycan-rich glial scar, which limits the regenerative capacity of the tissue. The degradation of the scar has therefore been suggested as a therapeutic strategy to improve local plasticity and promote axonal regrowth.

The bacterial enzyme chondroitinase ABC (ChABC) can degrade chondroitin sulfate proteoglycans, a major component of the glial scar, and has been proposed as a therapeutic strategy for multiple disorders. Yet, its inherent thermal instability, characterized by a rapid loss of activity at physiological temperatures, limits its therapeutic potential. Moreover, prolonged delivery is needed to obtain significant improvements after stroke.

We previously reported an affinity-controlled delivery platform for ChABC, in which the enzyme is expressed as a fusion protein with a Src homology-3 (SH3) domain and encapsulated in an injectable cross-linked methylcellulose hydrogel modified with SH3 binding peptides.[1] In order to address the intrinsic instability of ChABC, we designed a thermo-stable, 37 mutation, ChABC (ChABC-37-SH3) using computational remodeling and then tested it experimentally.[2] The ChABC-37-SH3 demonstrated a 6.5 times longer half-life than the native enzyme, with a higher melting temperature, and increased activity for its substrate. We are now, for the first time, evaluating a minimally invasive, sustained release strategy of this thermo-stabilized enzyme in vivo, using an endothelin-1-induced stroke injury model in rats. The in vivo efficacy of this strategy will be described in terms of tissue benefit, including mechanism of action and degradation of the glial scar.

Keywords

Stroke; glial scar; immunomodulation

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Abstract **#2009**

INTERFACIAL PHENOMENON AND NANOMECHANICS OF WOUND DRESSING HYDROGELS

Praveena Manimunda[P][1], Douglas Stauffer[1]

[1] *Bruker Nano Surfaces*

Corresponding author's email: Praveena.Manimunda@bruker.com

Hydrogels have become an integral part of regenerative medicine and tissue engineering.[1] The high-water content of hydrogels can present an exemplar atmosphere for cell survival, and the three-dimensional (3D) networks of cross- linked hydrophilic polymers provide the much-needed mechanical strength. Hydrogels are also used as a wound dressing material. They provide hydration to the wound cite and absorb exudates and can be applied as a permanent or temporary dressing material. Along with biocompatibility, stiffness of the hydrogels determine sensitization. As the healing of the injured epidermis starts, the mechanical properties, especially stiffness of the hydrogels are expected to vary. In this study, a new methodology was adopted to probe the mechanical property gradients in wound dressing materials. In situ confocal imaging in combination with nanoindentation was used to probe the local mechanical property variations in polyethylene glycol (PEG) hydrogels. Further the methodology was extended to study the stiffness changes at different loading rates and hydration levels.

Keywords

In Situ imaging, ; Wound Dressing

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Abstract **#2010**

ASSESSING THE ROLE OF BRANCHED CHAIN AMINO ACIDS IN ATHEROSCLEROSIS USING A HUMAN VASCULAR MICROPHYSIOLOGICAL SYSTEM

George Truskey[P][1], Ellery Jones[1], Svati Shah[2,3], Robert McGarrah[2,3]

[1] *Department of Biomedical Engineering, Duke University*

[2] *Department of Medicine, Duke University School of Medicine*

[3] *Duke Molecular Physiology Institute, Duke University School of Medicine*

Corresponding author's email: gtruskey@duke.edu

Metabolomic studies show that elevated serum levels of the branched chain amino acids (BCAA) leucine, isoleucine, and valine are associated with increased risk of cardiovascular and metabolic diseases. While in vitro experiments have shown that elevated leucine levels inhibit endothelial autophagy, induce oxidative stress, and inhibit nitric oxide release, these levels are 50-75 times higher than those observed in vivo during disease. To test the hypothesis that elevated BCAA plus a pro-oxidative environment induce endothelial dysfunction and increase the development of atherosclerosis, we perfused human tissue engineered blood vessels (TEBVs) with normal BCAA media for 7 days at a shear stress of 0.4 Pa. Vasoconstriction was measured after 1 µM phenylephrine exposure and vasodilation was measured after 1 µM acetylcholine exposure. After 7 days of perfusion, TEBVs were treated with 1x106 monocytes/ml in new media and the following of 4 treatment conditions: normal or elevated (5X higher than normal levels) BCAA media, with or without 50 µg/ml oxidized low density lipoprotein (oxLDL). After 48 h perfusion with the various treatments, no significant effect of oxLDL or BCAA was observed on vasoconstriction. Two-way analysis of variance indicated that elevated BCAA and oxLDL interact to significantly reduce endothelial-mediated vasodilation (**p=0.0087). Monocyte accumulation is greater in the elevated BCAA media with 50 µg/ml oxLDL than in normal BCAA media with 50 µg/ml oxLDL, suggesting that the combination of oxLDL and elevated BCAA can cause EC dysfunction and accelerate events in atherosclerosis.

Abstract **#2011**

MODELING SKELETAL MUSCLE FIBROSIS USING A HUMAN MICROPHYSIOLOGICAL SYSTEM

George Truskey$_P$[1], Anandita Ananthakumar[1]

[1] *Department of Biomedical Engineering, Duke University*

Corresponding author's email: gtruskey@duke.edu

diabetes, sarcopenia, and rheumatoid arthritis and is characterized by scarring, which impairs muscle contractile function and causes severe pain, affecting an individual's range of motion and quality of life. Muscular fibrosis is attributed to excessive deposition of extracellular matrix (ECM) in the skeletal muscle. We used human skeletal muscle engineered muscle bundles (myobundles) to examine factors which can promote loss of muscle function. To examine fibrosis, we matured myobundles for 7 days, added 1, 5 and 10 ng/ml transforming growth factor beta (TGF-beta) for 3 days, and measured mechanical properties and active contractile force. The tetanus force was significantly reduced at all concentrations of TGF-beta for 3 different donors. Interestingly, the bundle elastic modulus only increased at 10 ng/ml TGF-beta for 2 of the donors. This higher stiffness was associated with increased production of collagen 1,4 and 5 and fibronectin determined by RT-PCR and immunofluorescence. Fibroblasts were confined to the periphery and did not increase in number after TGF-beta treatment. An inhibitor (SB525334) of the downstream Smad pathway reduced the inhibitory effect of TGF-beta on force and the increase in collagen production. Interestingly, the inhibitor increased force in control myobundles not exposed to TGF-beta, which is consistent with measured secretion of TGF-beta by myobundles. These results suggest that TGF-beta induces fibrosis, altering both the passive and active mechanical behavior of skeletal muscle. Inhibiting steps in Smad signaling can restore function, opening possible treatment options.

Abstract **#2012**

VAPOR DEPOSITION TO CONSTRUCT PARTICLES AND SCAFFOLDING MATERIALS FOR REGENERATIVE MEDICINE

Hsien-Yeh Chen[P][1]

[1] *National Taiwan University*

Corresponding author's email: hsychen@ntu.edu.tw

Functionalized poly(p-xylylenes) can be deposited via chemical vapor deposition (CVD) polymerization to generate ultra thin films as conformal coatings and, due to the pre-defined chemical functionalities, provide a flexible solution to surface engineering challenges as they decouple surface design from bulk properties. Hence, the technology comprises essentially a one-step coating procedure to generate functionalized surfaces without requiring any kind of post-treatment once the films are deposited. Recently, three-dimensional porous structures are constructed via vapor deposition onto a sublimating solid template. Construction upon deposition of vapor-phase material occurs at a dynamic vapor–solid interface and is directed by the solid surface vanishing by sublimation. A proof-of-concept demonstration showed vapor depositions of poly-para-xylylenes on sublimating templates, including ice and mixtures with ethanol and hexane. The material construction macroscopically produces a replica architecture of the parent template. Characteristics of the pore structures are formed during the construction process as a result of the gas vapor and the space that is vacated by sublimation, thus enabling control of the porosity through regulation of the sublimation speed and/or the thermodynamic properties of the templates. The technology introduced herein provides a novel approach for 3D porous material manufacturing and overturns the notion that vapor deposition necessarily forms dense thin films on substrates. Applications of using the vapor sublimation and deposition process were demonstrated for the productions of hierarchical micro- and nano-particles and shaped scaffolding materials with customizable interface and bulk properties, and potentials of applying these materials for regenerative medicine were also highlighted.

Keywords

Tissue Engineering; Porous Polymer; Vapor Fabrication

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Abstract **#2013**

SUSTAINED EXPRESSION OF RUNX-1 ANABOLIC FACTOR IN MSCS BY CRE/LOXP BACULOVIRAL VECTOR FOR DISC DEGENERATION DISEASE THERAPY

Chin-Yu Lin[P][1], Long Yi Chan[2], Shu-Jui Kuo[3,4], Horng-Chaung Hsu[5,4]

[1] *Institute of New Drug Development, College of Medicine, China Medical University, Taichung, Taiwan*

[2] *Institute of New Drug Development, College of Medicine, China Medical University, Taichung, 40402, Taiwan.*

[3] *Department of Medicine, College of Medicine, China Medical University, Taichung, 40402, Taiwan.*

[4] *Department of Orthopaedics, China Medical University Hospital, Taichung, 40447, Taiwan*

[5] *Department of Medicine, College of Medicine, China Medical University, Taichung, 40402, Taiwan*

Corresponding author's email: geant@mail.cmu.edu.tw

Spinal disc degeneration is the most frequent factor associated with the inexplicable low back and neck pain, and accounted for the pain resulted disability worldwide. On current stage, available treatments for disc degeneration disease (DDD) are mostly symptomatic, and no single treatment has been found to directly control intervertebral disc (IVD) cell homeostasis. We hypothesize that if introduce a MSC source expressing cartilage-anabolic factors in the traumatic cavity may stimulate the mucoid materials secreted from nucleus pulposus and maintain the hydration content providing mechanical strength to decelerate the disc degeneration progression. In this study, a cartilage-anabolic factor runx-1 was expressed by baculoviral vector (BV) transduced MSCs through a Cre/LoxP gene recombination system for sustained recombinant protein production. Baculoviral vector possesses highly safety on the gene transduction in mammalian cells, can be manipulated in the biosafety level 1 facilities. The Cre/LoxP recombinant BV genetically modified MSCs were delivered by hyaluronan hydrogel encapsulation and transplanted to a coccygeal disc punctured model in rat through micro-injection, followed with x-ray radiographic examination, MRI-T2 hydration content evaluation and histological analysis at the end-point of 8-weeks post-transplantation. Our data revealed the Cre/LoxP recombinant BV system mediated long-termed runx-1 gene expression scenario, possessing good biosafety concern in the in vitro cell transduction and in vivo MSCs transplantation, and maintained superior hydration content in the disc than that of non-treatment. This methodology would fulfill the need of implanting therapeutic cells accompanied with discography, and could be further promoted to pre-clinical translational study in a large mammalian animal model.

Abstract **#2014**

ENGINEERED 3D HUMAN BONE MARROW MODELS

Dilara Perver[P][1], Viola Vogel[1]

[1] *Laboratory of Applied Mechanobiology, Institute of Translational Medicine, ETH Zürich, Switzerland*

Corresponding author's email: dilara.perver@hest.ethz.ch

The generation of sophisticated tissue structures with biological fidelity and organization is no longer science fiction, and human bone marrow (BM) is no exception.

Driving needs of timely available and targeted production of human hematopoietic stem and progenitor cells (HSPCs) required by transplantation therapies have fostered the BM-tissue engineering applications to unravel the ongoing debate on the niche-centric versus stem cell-centric vies of hematopoiesis and to use the outcoming cues for HSPC expansion. However, the challenges of BM-mimicry due to modeling constraints associated with its complexity still exist, and an adequate in vitro BM model providing a powerful platform for basic science and the clinical translation is still lacking. In fact, many existing tissue-engineered BM models cannot restate all the BM features at once. The models that have achieved the highest mimicry fidelity cannot bypass the use of animals. Besides, translation of those models to expand clinically relevant HSPCs with sufficient quality and amount is still a significant problem.

In this study, we first aimed at closing this gap by developing a novel xeno-free 3D BM model where the structural and compositional compartmentalization of BM is mimicked successfully, enabling the maintenance of HSPCs with functional properties. Further, this model is proved to be one of the most potent candidates to mimic the medullary cavity and host HSPCs, which allowed us successfully study HSPCs regulation over selected BM niche components with a high translational relevance by challenging the current methods of the widely applied 2D and gold-standard 3D suspension cultures.

Abstract **#2016**

ISL1 NICHES IN THE HUMAN FETAL HEART – IMPLICATIONS FOR REGENERATIVE MEDICINE

Shannon L Layland$_P$[1,2], Simone Liebscher[1,2], Ali Nsair[3], Katja Schenke-Layland[1,2,4,3]

[1] *Department of Women's Health, Research Institute for Women's Health, Eberhard Karls University Tübingen, Tübingen, Germany*

[2] *Department of Bioengineering, Eberhard Karls University Tübingen, Tübingen, Germany*

[3] *Department of Medicine/Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA*

[4] *NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*

Corresponding author's email: shannonlayland@yahoo.com

Insulin gene enhancer protein (ISL1) is a nuclear marker, expressed on neuronal and cardiac progenitor cells. Regenerative therapies have targeted ISL1+ cell surface markers for cell isolation and implantation. Innervating cardiac ganglia can be identified by ISL1. ISL1+ cells of cardiac and neurological lineage co-locate in similar areas of the developing mammalian heart. These findings present the question of whether ISL1 identifies cardiac progenitor cells, cardiac ganglia or both in humans. Proper identification of ISL1+ cells could determine their application in the areas of myocardial regeneration or cardiac electrophysiology. In this study, we isolated ISL1+ clusters from human 1st and 2nd trimester hearts. Immunofluorescence (IF) staining of cardiac tissues identified two distinct cluster types (C1) (C2) and the sinoatrial node as ISL1+ containing tissues. ISL1+ tissues were isolated using laser capture microdissection. Next Generation Sequencing (NGS) and proteomics analysis were performed, as well as Raman analysis and IF staining for neuronal and cardiac markers. NGS and proteomics data were analyzed for pathway enrichment using the Ingenuity Pathway Analysis software. Raman analysis showed that C1 and C2 ISL1+ cells have a very similar molecular profile when compared to SAN ISL1+ cells; however, C1 1st trimester and C1 2nd trimester differed, which was seen with C2 as well. NGS and proteomics analysis showed that C1 and C2 enriched predominantly neural pathways when compared to the SAN, which enriched cardiac pathways. This data suggests that ISL1 plays a larger role in the innervation of the developing heart; however, considerably more work is required.

Keywords

ISL1; heart; development

Abstract **#2017**

SYNTHETIC 3D MATRIX UNVEILS THE ROLE OF STRESS STIFFENING AND AVB3 INTEGRINS IN BONE FORMATION

Jung Un (Ally) Choi[P][1], Amanda W Kijas[1], Jan Lauko[1], Alan E Rowan[1]

[1] *Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia*

Corresponding author's email: jung.choi@uq.edu.au

Bone is constantly remodelled by bone-forming osteoblast and bone-resorbing osteoclast activities. This process is primarily regulated by the most abundant bone cells called osteocytes which they are able to sense their surrounding environment in the extracellular matrix (ECM) via αVβ3 integrins, localised along the dendritic cell processes. Despite the importance of matrix-driven cellular responses in bone homeostasis, osteocytes were understudied due to the lack of appropriate in vitro model. By employing αVβ3-integrin specific ligand in a tuneable synthetic biomimetic hydrogel ECM, we can interrogate individual parameters of ECM ligand adhesion and mechanical properties to establish in vivo like osteocyte morphology. We demonstrate that αVβ3 integrin-mediated adhesions in osteocytes promote bone formation by down-regulating key osteocyte-specific signalling proteins. Furthermore, the level of key secreted signalling molecules can be controlled by mechanical stress-stiffening properties of the synthetic matrices. This model represents the first physiologically relevant model for investigating this master regulator of bone homeostasis.

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Abstract **#2018**

CHARACTERISATION OF A NOVEL ASPECT OF TISSUE SCARRING FOLLOWING EXPERIMENTAL SPINAL CORD INJURY AND THE IMPLANTATION OF BIOENGINEERED TYPE-I COLLAGEN SCAFFOLDS IN THE ADULT RAT: INVOLVEMENT OF PERINEURIAL-LIKE CELLS?

Gary Anthony Brook$_P$[1], Pascal Achenbach[2,1], Sebastian Hammes[3,1], Moniek Palm[1], Istvan Katona[1], Ronald Deumens[4,1], Emmanuel Hermans[5], Hans Clusmann[6], Joachim Weis[1], Haktan Altinova[6,1,7]

[1] *Institute of Neuropathology, RWTH Aachen University Hospital*

[2] *Department of Neurology, RWTH Aachen University Hospital*

[3] *Department of Surgery, RWTH Aachen University Hospital*

[4] *Bernardinus College, Heerlen, The Netherlands*

[5] *Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium*

[6] *Department of Neurosurgery, RWTH Aachen University Hospital*

[7] *Police Headquarters Berlin, Medical Commission, Berlin, Germany*

Corresponding author's email: gbrook@ukaachen.de

Numerous intervention strategies have been developed to promote functional tissue repair following experimental spinal cord injury (SCI), including the bridging of lesion-induced cystic cavities with bioengineered scaffolds. Integration between implanted scaffolds and the lesioned host spinal cord is pivotal for the bridging function of such implantable scaffolds. Here, morphological studies have been conducted on 2mm long resection injuries of the adult rat cervical spinal cord lateral funiculus, followed by simple dural closure or the implantation of a microstructured type-I collagen scaffold. Immunohistochemistry revealed that, at 10 weeks post injury/implantation, relatively little integration could be observed between the scaffold and the surrounding host spinal tissues, as indicated by the paucity of regenerating axonal profiles or astrocytic processes within the scaffold. This was associated with the appearance of thin sheets of tightly packed and intensely zona occludens-1 immunoreactive fibroblast-like cells along the inner dura mater surface of control animals, and also forming a barrier around the implanted collagen scaffold. Transmission electron microscopy demonstrated uniform ultrastructural features including numerous tight junctions and caveolae at the plasma membrane, as well as a partial basal lamina surrounding the tightly packed cells. These striking ultrastructural features and the anatomical continuity of the sheets of scarring cells with local, damaged spinal nerve roots strongly suggest their identity to be migrating perineurial-like cells. This novel aspect of the cellular composition of reactive tissues highlights the unexpectedly complex nature of fibroadhesive scarring to traumatic SCI as well as to the implantation of bioengineered collagen scaffolds.

Keywords

Spinal cord injury; microstructured collagen scaffold; scarring response

Abstract **#2019**

INFLUENCE OF PERACETIC ACID STERILISATION ON THE BIOMECHANICAL PROPERTIES OF HUMAN TENDONS

Volker Eras[P][1], Kassandra Hoetzel[1], Jan Brune[1]

[1] *German Institute for Cell- and Tissue Replacement, DIZG gGmbH - Non-Profit (Berlin)*

Corresponding author's email: n_ahmed@dizg.de

Human allograft tendons are used in a variety of indications in the fields of sports medicine, foot and ankle as well as trauma surgery. Peracetic acid (PAA) sterilisation has been shown to preserve tissue biomechanics of bone-patella tendon-bone constructs [1]. This study aims to evaluate the influence of PAA-sterilisation on the biomechanical properties of human tendons.

Pairs of tibialis anterior (TA) and posterior (TP) tendons were explanted and frozen according to tissue banking standards. For each pair, one tendon was sterilised in PAA leaving the contralateral tendon frozen. Tensile tests were performed on an InspektTableBlue (H&P, Germany). Samples were looped around bolts and cryoclamped on both ends while the midsubstance of the tendons remained free, adhering to material testing standards. Tendons received a 10-cycle preconditioning between 50 N and 250 N, a 5-minute load relaxation and a failure test with 50 mm/s speed.

Out of 27 donors tested, tendons of nine pairs (TP: 5; TA:4) exhibited a valid failure mode of rupture at the midsubstance and hence were included in the analysis. There was no significant difference between PAA treated and fresh frozen (FF) TA and TP tendons in terms of failure load (PAA: 2557 ± 401 N vs. FF: 2394 ± 614 N). Additionally, both types of treatment revealed similar load relaxation behaviour (PAA: 17.0 ± 4.2 % vs. 18.4 ± 5.9 %).

PAA-sterilisation does not negatively influence biomechanical properties of human tendons in terms of relaxation and failure loads allowing for a safe and biomechanically reliable graft.

Keywords

allograft; tendons

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Abstract **#2020**

ABSORPTION CAPACITY OF BONE-BASED CARRIER-GRAFTS

Jan Brune[P][1], Anja Hanke[1], Volker Eras[1], Norus Ahmed[1]
[1] German Institute for Cell- and Tissue Replacement, DIZG gGmbH - Non-Profit (Berlin)
Corresponding author's email: n_ahmed@dizg.de

Delivery of cells and expensive growth-factor containing liquids to their intended site of action is difficult as these transferred cells and fluids are easily washed away during surgery and often removed via homeostasis [1]. Future treatments involving cell therapy and tissue engineering products mandate suitable carriers for efficient retention of cells and fluids in situ.

Here, we analysed human tissue scaffolds including specifically designed high absorption and high surface area carrier-grafts, for their interstitial and matrix-linked absorption capabilities.

Eight groups of carrier grafts were analysed. Two groups of high surface area cortical fiber grafts, demineralized bone matrix (1–2 mm), cortical granules (1–2 mm), three different densities of mineralized cancellous bone and demineralized cancellous bone. All were rehydrated with phosphate-buffered saline (PBS) to a final volume of 0.4 cc.

Total absorption – both demineralized cancellous bone (0.844 ± 0.083 ml/cc) and cortical fibers (0.599 ± 0.025 ml/cc) absorbed a significantly larger volume of total PBS compared to the amount absorbed by cortical granules (1-2 mm; 0.376 ± 0.042 ml/cc).
Matrix absorption – The largest absorption capacity of the tissue matrix was observed in the cortical fibers (88.82 ± 2.99%) while low-density cancellous bone displayed the smallest matrix-linked absorption capacity (0.95 ± 1.07%).
Interstitial spaces – Investigating the uptake of PBS into interstitial spaces, revealed that demineralised cancellous bone absorbed the largest amount of PBS into the Interstitial spaces (84.4 ± 3.84%).

Cortical fibers displayed high absorption capabilities and may provide as a valuable addition to the regenerative medicine toolkit.

Keywords
bone; allograft

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Abstract **#2021**

CELLULOSE-BASED SCAFFOLDS ENHANCE PSEUDOISLETS FORMATION AND FUNCTIONALITY.

Júlia Rodríguez-Comas$_P$[1], Ferran Velasco-Mallorquí[1], Javier Ramón-Azcón[1,2]

[1] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST). 08028, Barcelona, Spain.*

[2] *Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain*

Corresponding author's email: jrodriguezc@ibecbarcelona.eu

The limitations of obtaining pancreatic islets from different sources as animal models or human donors complicate the study of type 2 diabetes (T2D) in vitro. Immortalized cell lines as the insulin-producing INS1E β-cells appeared as a valid alternative to model insulin-related diseases. The formation of 3D structures to promote cell aggregations from single cells is a handy tool to generate resemblance islet-like pseudoislets. Traditionally used hydrogel encapsulation methods induce a lack of nutrient and oxygen diffusion for pancreatic tissue engineering. Here, we use cryogelation technology to develop a more resemblance scaffold with the mechanical and physical properties needed to engineer pancreatic tissue. This study shows that carboxymethyl cellulose (CMC) cryogels prompted cells to generate β-cell clusters. The high porosity achieved with this approach allowed us to create specific range pseudoislets. However, gelatin-based scaffolds did not induce this cell organization. Pseudoislets formed within CMC-scaffolds showed cell viability for up to 7 days and responded better to the glucose over conventional monolayer cultures. Overall, our results demonstrate that CMC-scaffolds can be used to control the organization and function of insulin-producing β-cells, representing a suitable technique to generate β-cell clusters to study pancreatic islet function.

Keywords

Diabetes; Cryogel; Cellulose

Abstract **#2022**

LAYERED DOUBLE HYDROXIDE NANOPARTICLE-REINFORCED BONE TISSUE ENGINEERING SCAFFOLDS

Zeynep Puren Akguner$_P$[1], Ayca Karagoz Koroglu[2], Hakan Darici[2], Erdal Karaoz[2], Ayca Bal Ozturk[3]

[1] *Department of Stem Cell and Tissue Engineering, Institute of Health Sciences, Istinye University, Istanbul, Turkey*

[2] *Department of Histology and Embryology, School of Medicine, Istınye University, Istanbul, Turkey*

[3] *3 Department of Analytical Chemistry, Faculty of Pharmacy, Istinye University, Istanbul, Turkey*

Corresponding author's email: zeynepakguner@gmail.com

The current clinical routines such as autograft and allograft transplantation have drawbacks for healing bone defects, mostly due to the limited convenient sources, donor rejection, and risk of infection [1]. Tissue engineering strategies are innovative and remarkable that utilize the potential of bone progenitors, especially stem cells, to differentiate into functional bone cells. Scaffolds have an important function in tissue engineering application, they not only contribute as structural support for specific cells but also contribute as a matrix to guide new tissue growth. In the present study, the feasibility of fabricating Mg-Al layered double hydroxide (LDH)- reinforced GelMA/Silk fibroin-based nanohybrid scaffolds was explored. Verifying the mineralization and differentiation potential of stem cell-laden nanohybrid scaffolds was aimed. For this purpose, firstly, stem cell-laden scaffolds were prepared by micro-molding technique under the UV light. After the 14 and 21 days of culture, cell viability, proliferation potential, metabolic activity, and ALP activiy were evaluated and analyzed. Alizarin Red, Hematoksilen & Eosin, Masson Trichrome, and immunofluorescence stainings were performed. Our findings confirmed the use of LDH nanoparticle- reinforced nanohybrid scaffolds as a composite matrix for bone tissue engineering applications, being capable of supporting natural bone regeneration as well as mineralized- matrix deposition and triggering a functional bone tissue regeneration.

This work was suporrted by The Scientific and Technological Research Council of Turkey (TUBITAK) [Project Number: 118S549].

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Abstract **#2023**

MICROFLUIDICS-ENHANCED BIOPRINTING FOR TISSUE FABRICATION

Y. Shrike Zhang[P][1]

[1] *Harvard Medical School*

Corresponding author's email: yszhang@research.bwh.harvard.edu

Over the last decades, the fabrication of three-dimensional (3D) tissues has become commonplace. However, conventional 3D fabrication techniques are limited in their capacity to produce complex tissue constructs with the required precision and controllability that are needed to replicate their native counterparts. To this end, 3D bioprinting offers great versatility in the fabrication of biomimetic volumetric tissues that are structurally and functionally relevant. It enables accurate control of the composition, spatial distribution, and architecture of bioprinted constructs facilitating the recapitulation of the delicate shapes and structures of target organs and tissues. This talk will primarily discuss our recent efforts in developing microfluidics-enhanced bioprinting methods that enable unconventional bioprinting applications not quite possible or convenient in traditional approaches. These platform technologies are likely to provide new opportunities in constructing functional tissues to facilitate regeneration, as well as in generating microtissue models for promoting personalized medicine.

Abstract **#2024**

THE ROLE OF 3D PRINTED BARRIER CONSTRUCT FOR BONE ENGINEERING FOR SYSTEMIC INFLAMMATORY CONDITIONS

Adil Akkouch[P][1,2], Zeena Qiryaqoz[3], Robert Swenson[4], Kim Brogden[5], Liu Hong[5]

[1] *Department of Orthopaedic Surgery, Western Michigan University Homer Stryker MD School of Medicine*

[2] *Medical Engineering Program, Western Michigan University Homer Stryker MD School of Medicine*

[3] *Medical program, Western Michigan University Homer Stryker MD School of Medicine*

[4] *Department of Periodontics, College of Dentistry, The University of Iowa*

[5] *Iowa Institute for Oral Health Research, College of Dentistry, The University of Iowa*

Corresponding author's email: adil.akkouch@med.wmich.edu

Increased fracture rates and nonunion defects occur in several chronic inflammatory conditions (1). Delayed bone healing can be reversed by the inhibition of inflammatory cytokines and by enhancing osteoblast activity (2), however, this process is too complicated and involves a plethora of biomolecules and pathways. Controlling the microenvironment of the bone defect may be more efficient and feasible strategy. Guided Bone Regeneration uses membranes that act as a barrier to prevent soft-tissue invasion and to promote bone regeneration. However, in large defects, bone formation occurs only to the marginal zone and use of grafting materials is required; which limited their use in orthopedic surgeries. In this study, we propose the use of a 3D bioprinted construct made by mineralized collagen and pre-osteoblast cells as barrier construct.
Methods: We prepared constructs of collagen and hydroxyapatite and HEPM cells using 3D bioprinting. We used histology, SEM, TEM and mechanical testing to characterize the structure. Constructs were incubated with cytokines to measure adsorption. The production of chemokines and cytokines was assessed.
Results: The fabrication of the 3D construct using 3D bioprinting was not toxic and did not alter the viability, proliferation and cytokines expression of HEPM cells. Constructs served as a barrier and did not absorb any of the cytokines measured GM-CSF, IL-12, IL-1α, IL-6, IL-8, MIP-1b, RANTES, or TNF-α.
Conclusion: The 3D construct is osteoconductive and act as a 3D barrier to surrounding anti-osteogenic mediators.This barrier can be used in patients with chronic inflammation conditions.

Keywords

Barrier membranes; Guided bone regeneration; Bioprinting

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Abstract **#2025**

A PLUG WITH SHAPE MEMORY TO SEAL IATROGENIC FETAL MEMBRANE DEFECTS AFTER FETOSCOPIC SURGERY

Rob T C Meuwese[P][1], Elly M M Versteeg[1], Joris van Drongelen[2], Daniëlle de Hoog[1], Debora Bouwhuis[1], Toin H van Kuppevelt[1], Willeke F Daamen[1]

[1] Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Radboud university medical center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands

[2] Department of Obstetrics & Gynaecology and Dept. of Neonatology, Radboud university medical center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands

Corresponding author's email: rob.meuwese@radboudumc.nl

Iatrogenic preterm premature rupture of membranes (iPPROM) is seen as the Achilles' heel of minimal invasive fetal surgery as it is a strong trigger for premature birth, subsequently increasing the risk of morbidity and mortality for the baby. As fetal membrane defects do not heal spontaneously, we developed a biocompatible, fetoscopically applicable collagen plug with shape memory, which expands directly upon employment by an entropy-driven force to seal the fetal membranes, hence preventing amniotic fluid leakage and plausibly iPPROM.

Plugs were constructed by a process consisting of casting, freezing, and lyophilization of a type I collagen suspension. Lyophilized collagen plugs were given shape memory and fitted through the fetoscope shaft (ø3.3 mm). Expansion of the plug was examined in PBS. The quality of sealing was studied ex vivo using human fetal membranes, and using a high pressure in situ porcine bladder model.

The crimped plug expanded to the energetically favorable state, and tripled in diameter within 1 min when placed into PBS, so it will be able to fill the entire endoscopic entry point. In comparison, collagen plugs without shape memory did not expand beyond 3 mm within 1 hour. In both human fetal membranes and a porcine bladder, the plug expanded in the defect, secured itself and sealed the defect. No substantial leakage nor rupture were observed. In conclusion, a collagen plug with shape memory is a promising medical device that can rapidly seal a fetoscopic defect in fetal membranes at the endoscopic entry point, and may prevent iPPROM.

Keywords

Fetoscopic surgery; Fetal membranes; Medical device

Abstract **#2026**

TREATMENT OF PORCINE FULL-THICKNESS WOUNDS BY TRANSPLANTATION OF BIODEGRADABLE MICROCARRIERS CARRYING AUTOLOGOUS FIBROBLASTS AND KERATINOCYTES

Shora Zamani Fekri[1], Alexander Larsson[1,2], Victor Hanna[1], Karin Gustafsson[1], Jonathan "Rocket" Rakar[1,3], Hans Vintertun[1,2], Gunnar Kratz[1,2], Johan Junker[1,3]

[1] *Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden.*

[2] *Department of Hand Surgery, Plastic Surgery and Burns, Linköping University Hospital, Linköping, Sweden.*

[3] *The Center for Disaster Medicine and Traumatology, Linköping University Hospital, Linköping, Sweden.*

Corresponding author's email: johanjunker@gmail.com

Skin grafting is standard treatment of extensive wounds, despite frequently resulting in poor cosmetic and functional outcome. To mitigate this problem, tissue engineered skin substitutes comprised of a combination of cells and biomaterials have been suggested. Rapid cell expansion and subsequent transplantation is desirable, preferably without the use of enzymes. The present study aimed at investigating transplantation of keratinocytes and fibroblasts seeded on microcarriers in a porcine full-thickness wound model.

Cells were isolated from skin biopsies taken from pigs (N = 8), expanded, and seeded on Porcine Gelatin Microcarriers. Resulting tissue constructs were transplanted to 120 surgical wounds (2 cm diameter). Treatment groups included NaCl control, microcarriers, and microcarriers seeded with fibroblasts, keratinocytes or both. Wounds were excised after one, two, four or eight weeks for histological and immunohistochemical analysis. All animal procedures were approved by the Regional Ethical Review Board (protocol ID534).

Quantifying cells on microcarriers revealed optimal cell density 24-96h after seeding. Following transplantation, microcarriers were incorporated into the granulation tissue, with complete degradation after two weeks. The first two weeks after transplantation, microcarriers seeded with cells resulted in significantly thicker neoepidermis compared to microcarriers and NaCl controls ($p < 0.05$). Four and eight weeks after transplantation, no significant differences could be observed.

In conclusion, experiments performed illustrate the successful use of PGMs as a biodegradable cell carrier for transplantation of fibroblasts and keratinocytes. The proposed method may be useful for cell expansion and transplantation, enabling delivery of large numbers of cells without the use of enzymes.

Abstract **#2027**

HUMAN KERATIONCYTES CHANGE THEIR GENE EXPRESSION WHEN STIMULATED WITH AMNIOTIC FLUID - IMPLICATIONS FOR WOUND HEALING PROCESSES

Elvira Lindholm$_P$[1], Erika Nyman[1,2], Jonathan "Rocket" Rakar[1,3], Johan Junker[1,3], Gunnar Kratz[1,2]

[1] *Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden.*

[2] *Department of Hand Surgery, Plastic Surgery and Burns, Linköping University Hospital, Linköping, Sweden.*

[3] *The Center for Disaster Medicine and Traumatology, Linköping University Hospital, Linköping, Sweden.*

Corresponding author's email: johanjunker@gmail.com

Early fetal wounds heal with regeneration instead of scarring and amniotic fluid (AF) containing hyaluronic acid (HA) is thought to be an important factor for this. In this study we aimed to analyze the gene expression in keratinocytes during stimulation with human AF.

Human primary keratinocyte cultures were serum-starved for twelve hours, then cultured in DMEM alone or with 50% AF or with 50% FCS. RNA was collected and pooled and gene expression was analyzed using Affymetrix U133A plus 2 microarray with enrichment analysis performed in Enrichr and WebGestalt.

About 2,000 distinct genes were differentially regulated between DMEM and AF groups, and about 1,500 genes between FCS and DMEM groups. About 600 distinct genes were differentially regulated between AF and FCS. The AF and FCS treatments resulted in enrichment of terms relating to the epidermis and keratinocyte biology. In particular, p63, AP-1 and NFE2L2 (Nrf2) associated genes were found significantly regulated in both treatments. Genes regulated by FCS treatment were more associated with TNF, NFkB and inflammatory signaling while AF treatment was more associated with molecular establishment of epidermis, enhanced lipid metabolism and tissue architecture.

In conclusion, our results indicate that AF may trigger a differentiation program that includes a higher degree of regeneration than the adult wound bed supports which could explain the positive effects on human wound healing found in AF. This study might serve as a source for further understanding the potential therapeutic tole of AF in wounds that are reluctant to heal.

Abstract **#2028**

EX VIVO INFECTED WOUND MODEL BASED ON VIABLE HUMAN SKIN FOR TESTING NOVEL ANTIMICROBIAL TREATMENTS

Jonathan "Rocket" Rakar[P1,2], Kristina Hanna[1], Zeljana Sotra[1], Oskar Wikström[1], Elin Karlsson[1], Gunnar Kratz[1,3], Johan Junker[1,2]

[1] *Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden.*

[2] *The Center for Disaster Medicine and Traumatology, Linköping University Hospital, Linköping, Sweden.*

[3] *Department of Hand Surgery, Plastic Surgery and Burns, Linköping University Hospital, Linköping, Sweden.*

Corresponding author's email: johanjunker@gmail.com

The current threat of antibiotic resistance coupled with slow development of novel antimicrobial treatments calls for new methods for rapid and reproducible evaluation of potential therapeutics. The aim of the present study was to develop a standardized ex vivo infected wound model. The model was then used to evaluate antimicrobial treatment in regards to reduction of infection and reepithelialization.

Normal skin was obtained from patients undergoing routine surgery (Swedish Ethical Review Authority no. 2018/97-31). Full-thickness disks (8 mm diameter) with a central partial-thickness wound (4 mm diameter) were created using biopsy punches. The wounds were inoculated with 10E7 Staphylococcus aureus (ATCC 29213), and maintained under standard conditions. Tissue infection was monitored using quantitative cultures and immunohistochemical staining using antibodies against S. aureus and pancytokeratin. Topical gentamicin administration (1 mg/ml) was evaluated in regards to eradication of infection using the infected wound model.

Quantitative cultures revealed a wound infection three days after inoculation of approximately 10E7 colony forming units (CFU)/g of tissue, increasing to 10E9 CFU/g twelve days after inoculation. Immunohistochemical staining confirmed the bacteria to be S. aureus, and revealed the spread of infection down in to the dermis. Antimicrobial treatment using topical gentamicin completely eradicated the wound infection.

The current study describes the establishment of an infected wound model based on viable human skin. The model is reproducible and provides rapid screening of novel antimicrobial treatments, assessing reduction of infection as well as reepithelialization over time. Further development of the infected wound model will include other clinically relevant pathogens.

Abstract **#2029**

ZONAL DESIGN OF CARTILAGE IMPLANTS: MECHANOTOLERANCE OF CHONDROCYTES AS A SELECTION CRITERIUM FOR IMPLANT DESIGN

Janine Lueckgen[1], Elisabeth Kraemer[1], Tobias Reiner[2], Wiltrud Richter[1]

[1] *Research Center for Experimental Orthopaedics, Heidelberg University Hospital*

[2] *Department of Orthopaedic and Trauma Surgery, Heidelberg University Hospital*

Corresponding author's email: Janine.lueckgen@med.uni-heidelberg.de

Current cartilage replacement strategies often display mechanical failure, impeding proper joint function. A zonal design of implants for cartilage regeneration improved integration and mechanical stability over non-zonal approaches. Chondrocytes in the upper zone enable functionality of the cartilage layer and may be obtained in limited quantity from articular cartilage (AC) or rather unlimited from mesenchymal stroma cells (MSC) differentiated towards the chondrogenic lineage. So far it remains unknown, whether an MSC-derived chondrocyte layer tolerates mechanical loading equally well as an AC-derived zone and is, therefore, promising as upper zone of cartilage constructs. Aim of this study was to compare the mechano-response of engineered AC- and MSC-derived zonal implants to physiological loading.

Implants were generated by seeding human AC or MSC in a collagen I/III scaffold attached to a beta-TCP block. After 21 days of chondrogenic maturation, 3h of cyclic compression was applied in a bioreactor. Matrix synthesis, gene and protein-expression were analyzed.

MSC-derived cartilage reacted with a lower load-induced upregulation of multiple mechano-response genes and loading disturbed cartilage matrix synthesis which was not seen with AC. Load-induced BMP2- and BMP6-induction was higher in the AC-group, but BMP-stimulation of MSC during loading did not eliminate the disturbance of matrix synthesis. Differences in NFkB-signaling between AC and MSC suggested a link of the differential response to the NFkB-pathway.

In conclusion, MSC-derived osteochondral implants displayed a reduced tolerance to physiological loading compared to AC with disturbance of matrix synthesis, suggesting the cell-source for the cartilage layer of zonal implants should be carefully considered.

Abstract **#2030**

3D IN VITRO MODELS FOR THE STUDY OF LIVER-SKELETAL MUSCLE AXIS IN NAFLD

Ainhoa Ferret Miñana[P][1], Francesco De Chiara[1], Javier Ramón Azcón[2]
[1] *Biosensors for bioengineering group, Institute for Bioengineering of Catalonia (IBEC)*
[2] *Biosensors for bioengineering group, Institute for Bioengineering of Catalonia (IBEC), ICREA*
Corresponding author's email: aferret@ibecbarcelona.eu

Non-alcoholic fatty liver disease (NAFLD) affects 1 in 4 people worldwide. It ranges from simple steatosis to non-alcoholic steatohepatitis, which may progress to cirrhosis, and hepatocellular carcinoma. From 30 to 70% of patients with NAFLD suffer from generalised loss of skeletal muscle (SM) mass (sarcopenia). Why and how skeletal muscle mass influences the development of NAFLD is not completely elucidated. Here, we present a three-dimensional model of fatty liver and subsequent loss of SM in vitro.

Mouse hepatocytes and AML2 and SM C2C12 were encapsulated in solution of gelatin methacryloyl and sodium carboxymethylcellulose at concentration of 5 and 1%, respectively. The photo-initiator LAP was then added at concentration of 0.1% and the polymer exposed at UV light for 30 seconds. The fatty liver is induced upon incubation of the cell with non-esterified fatty acids (NEFAs) for various timepoints. The supernatant from those cells were then incubated with SM cells.

Hepatocytes showed lipid accumulation, nuclei distortion and cell death after 48h of culture with NEFAs assessed by confocal and bright microscopy. Albumin and urea cycle enzymes levels also showed a time dependent decrease at protein and mRNA levels. The SM cells in contact with supernatant from fatty hepatocytes displayed loss of cytoplasmatic mass, metabolic activity and efficiency in time dependent manner as showed by H&E staining and MTS assay, respectively.

Liver and SM are connected at cellular level during the development of NAFLD, pinpointing to a broader therapeutic approach to the disease.

Keywords
NAFLD; Skeletal muscle; Sarcopenia

Abstract **#2031**

MICROTECHNOLOGIES FOR COMBINATORIAL SCREENING OF STEM CELL MECHANOBIOLOGICAL RESPONSES IN 3D BIOMATERIALS

Craig A Simmons$_P$[1,2,3]

[1] *Translational Biology & Engineering Program, Ted Rogers Centre for Heart Research*

[2] *Department of Mechanical & Industrial Engineering, University of Toronto*

[3] *Institute of Biomedical Engineering, University of Toronto*

Corresponding author's email: simmons@mie.utoronto.ca

Cells are exquisitely sensitive to their biomaterial microenvironment, producing integrated responses to a myriad of biochemical and biophysical cues so as to direct tissue growth and physiology. This responsiveness can be leveraged in regenerative medicine therapies by providing the cells with the right cocktail of biochemical factors, biomaterial cues, and biophysical stimuli to guide their fate and function. Fully realizing this potential is challenging, however, because the effects of multiple interacting soluble and biophysical stimuli are complex and difficult to measure and optimize. To address this need, we have developed microfabricated deformable membrane platforms that uniquely enable dynamic mechanical stretch and biomechanical monitoring of arrayed 3D biomaterial constructs. We combine these platforms with statistical modeling to systematically probe multifactorial regulation of cell mechanobiological responses. In this talk, I will present our recent studies that use this strategy to define biomaterial design parameters and novel mechanical loading protocols that promote maintenance of stem cell pluripotency and tissue regeneration by mesenchymal stem cells.

Abstract **#2032**

UROGRAFT- A BIOCONFIGURABLE COMPOSITE GRAFT FOR URINARY BLADDER WALL REGENERATION

Marta Pokrywczynska[P][1], Jan Adamowicz[1], Marta Rasmus[1], Michal Stopel[2], Daria Balcerczyk[1], Zuzanna Fekner[1], Marta Szulc[3], Tomasz Kloskowski[1], Arkadiusz Jundzill[1]

[1] *Department of Regenerative Medicine, Cell and Tissue Bank, Chair of Urology and Andrology, Ludwik Rydygier Medical College, Nicolaus Copernicus University, Bydgoszcz, Poland*

[2] *Department of Mechanics and Computer Methods, University of Science and Technology, Bydgoszcz, Poland*

[3] *Department of Chemistry of Biomaterials and Cosmetics, Nicolaus Copernicus University, Torun, Poland*

Corresponding author's email: marta.pokrywczynska@interia.pl

Regenerative medicine has developed over last decades a new interdisciplinary approach to urinary bladder wall regeneration based on biomaterial scaffolds intended to activate intrinsic regeneration mechanisms. To date, urinary bladder reconstruction applying this strategy has been tested in 141 patients (1). Nevertheless, none of them was successfully translated into clinics due to high complication rate and lack of reliable long lasting function.

The aim of the study was to create and evaluate UROGRAFT- a new bioconfigurable, composite graft for urinary bladder wall regeneration.

The impact of the UROGRAFT configuration on its mechanical properties and potential for regeneration of reconstructed urinary bladder wall was assessed. For this purpose, a numerical modeling technique based on the Finite Element Method was used. Selected UROGRAFT configuration (30cm2) was used for augmentation cystoplasty in pigs (n=5). Bladder acellular matrix (BAM) in a circle shape (30cm2) was used for augmentation cystoplasty as a control (n=5). The follow-up of the study was 6 months. Reconstructed bladders were subjected to radiological, macroscopic, histological and molecular evaluations.

All animals survived the 6 months follow-up. Tissue engineered bladder function was normal without any signs of post voiding urine residual in bladder and in the upper urinary tracts. Macroscopically, bladder wall reconstructed with UROGRAFT mimicked native urinary bladder tissues. Graft shrinkage and fibrosis were observed in control group. Histological and molecular analysis confirmed that UROGRAFT enhanced regeneration of reconstructed bladder wall.

The present work was supported by the National Center for Research and Development (NCBR) in Poland under agreement no. LIDER/48/0195/L-9/17/NCBR/2018

Keywords

urinary bladder; augmentation cystoplasty; tissue engineered graft

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Abstract **#2033**

TRANSLATION OF A NERVE GUIDE FROM SMALL ANIMALS TO LARGE ANIMALS TO HUMANS

Kacey Marra[P][1]

[1] *University of Pittsburgh*

Corresponding author's email: marrak@upmc.edu

Severe injuries to peripheral nerves are challenging to repair. For nerve gaps >one inch, the standard of care is autografting; however, autografting can result in neuroma formation, loss of sensory function at the donor site, and increased operative time. To address the need for an off-the-shelf synthetic nerve conduit to treat large nerve gaps, we developed a biodegradable conduit comprised of poly(caprolactone), (PCL). The inner portion of the PCL nerve conduit was embedded with double-walled poly(lactic-co-glycolic acid)/poly(L-lactic acid), (PLGA)/(PLLA) microspheres encapsulating glial cell line-derived neurotrophic factor (GDNF). Functional, electrophysiological and histological assessments demonstrated the efficacious bridging of a long gap in a non-human primate 5-cm median nerve defect model. The next steps (e.g. clinical translation) for those in academia can be particularly challenging. This talk describes the 20-year journey from biomaterials fabrication and characterization, to in vitro cell studies, to small animal studies, to large animal studies, GMP manufacturing, ISO 10993 biocompatibility testing, regulatory approval, and clinical trials.

Abstract **#2035**

BUILDING MACRO-TISSUE ASSEMBLIES USING A SYNERGETIC TISSUE ENGINEERING STRATEGY

Olivier Guillaume$_P$[1,2], Aysu Arlan[3], Peter Dubruel[3], Sandra Van Vlierberghe[3], Aleksandr Ovsianikov[1,2]

[1] 3D Printing and Biofabrication Group, Institute of Materials Science and Technology, TU Wien, Vienna, Austria

[2] Austrian Cluster for Tissue Regeneration (www.tissue-regeneration.at)

[3] Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Ghent University, Belgium

Corresponding author's email: olivier.guillaume@tuwien.ac.at

Introduction:

The bottom up approach in the field of tissue engineering requires small and modular building block units which can ideally self-assemble together [1]. The work here represents an alternative option to create those building blocks by combining spheroids with micro-size polymeric scaffolds produced using multi-photon lithography (MPL).

Experimental methods:

Photo-polymerizable resin is based on a multifunctional acrylate-endcapped urethane-based poly(caprolactone) (AUP-PCL) [2] dissolved in THF with M2CMK at 10mM as photo-initiator. Micro-size scaffolds were printed using MPL, with a femtosecond laser at 800nm, 10x microscope objective, at intensities ranging from 20 to 300 mW at 1000 mm.s-1. Each microscaffold was incubated with a suspension of human-adipose derived stem cells (hASC), in agarose molds, until formation of spheroids. Morphological features along with the self-assembly property of the structures were assessed microscopically and compared with normal spheroids.

Results and Discussion:

Under low-binding condition, individual spheroids could be formed within each of the microscaffolds. Importantly, the presence of the polymeric structures permits the fabrication of tissues through self-assembly, superior in size, stability and control in terms of shape compared to conventional spheroid-based tissues. The next step is now to upscale the MPL fabrication and the spheroids sorting necessary to envision any bench-to-bed translation.

Conclusions:

Microscaffolds built using MPL combined with spheroids possess excellent properties to generate building blocks for large-size tissue self-assembly. This synergetic tissue engineering strategy offers unprecedented opportunities compared to scaffold-free and scaffold-based approaches, with great perspectives to reconstitute complex tissue defects [3].

Acknowlegments:

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Abstract **#2036**

MULTI-CELLULAR SPHEROID ENGINEERING USING BIOINSPIRED MATERIALS

Heungsoo Shin[P][1]

[1] *Hanyang University*

Corresponding author's email: hshin@hanyang.ac.kr

Recently, technologies to culture one or more cell types in 3D conditions have attracted a great deal of attention in tissue engineering. In particular, stem cell spheroids have been reported for improved viability, self-renewal capacity, and differentiation potential. However, for the use of multi-cellular spheroids in tissue engineering, modulation of spheroid functions with instructive signals is critical. We have been developing ECM-mimicking fibrous materials decorated with cell-instructive cues, which were incorporated within 3D stem cell spheroids to tune their functions as modular building blocks for bottom-up tissue-engineering applications. In this presentation, engineering ECM-mimicking materials and their use in 3D spheroid fabrication would be discussed with utilizing human-adipose-derived stem cells (hADSCs) with aims of enhanced bone regeneration.

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Abstract **#2037**

CURVATURE: A POTENT DYNAMIC REGULATOR OF CELL PHENOTYPE AND MIGRATION

Nicholas Kurniawan[P][1]

[1] *Eindhoven University of Technology*

Corresponding author's email: kurniawan@tue.nl

The intrinsic architecture of tissues subject cells to geometrical cues in the form of mesoscale curvatures. While the effect of nano- and micro-scale topographical cues have been extensively studied for decades, surprisingly little is known about the cellular response to substrate curvatures. To study the effect of curvature in a systematic and high-throughput manner, we developed "2.5D curvo-chips": microfabricated chips containing arrays of concave and convex geometrical structures with a wide range of sizes (µm to mm). We observed distinct cell adhesion morphologies on convex and concave structures that are accompanied by changes in the phosphorylated myosin, nuclear lamin-A, and F-actin levels, suggesting a compensation mechanism due to curvature-driven impairment of F-actin assembly to maintain cellular contractility. These resulted in distinct cell migration modes that depended solely on the instantaneous curvature 'perceived' by the cells. Furthermore, we developed two methods to simultaneously present competing curvature and contact-guidance cues to the cells. The results show a clear 'dose-dependent' curvature sensing by the cells. Intriguingly, repeating these experiments with a variety of cell types (fibroblasts, mesenchymal stromal cells, endothelial cells, epithelial cells, keratocytes) reveals that the ability of cells to sense and respond to mesoscale substrate curvatures is universal, but highly cell-type-specific. Our findings establish mesoscale curvature as a potent regulator of cell phenotype and behavior, and demonstrate a complex interplay between the dynamics of actin stress fiber (re)organization and force generation, nucleus mechanics, and adhesion morphology in dynamically guiding cell phenotype and migration behavior on physiologically-relevant 3D structures.

Abstract **#2038**

DECONSTRUCTING REGENERATIVE THERAPEUTICS: CELLS, EXTRACELLULAR VESICLES AND NONCODING RNA

Eduardo Marban[P][1]

[1] *Smidt Heart Institute, Cedars-Sinai Medical Center*

Corresponding author's email: eduardo.marban@cshs.org

The development of regenerative therapy has benefited greatly from serendipity. Cell therapies have some promise, but cell products vary in potency and can be difficult to manufacture. In the process of mechanistic investigations into cell therapies for heart disease, we discovered a central role for extracellular vesicles (EVs) as paracrine mediators of therapeutic bioactivity. EVs reproduce the benefits of cells, and they are easy to isolate, store and deliver, making them viable cell-free therapeutic candidates. But EVs may also have value as a discovery platform for biologically-active molecules. EVs work by delivering RNA and protein payloads to target cells, so it is logical to mine EV contents in a search for defined factors. In so doing we have found several noncoding RNA species, including fragments of Y RNA, which themselves have intriguing biological actions. The next generations of cell-free biologics (EVs and noncoding RNAs) may provide the benefits of cell therapy without the intrinsic limitations.

Abstract **#2039**

PHOTOBIOENGINEERING: TWO SYNERGISTIC BIOTECHNOLOGIES FOR REGENERATIVE MEDICINE

Praveen Arany[P][1]

[1] *University at Buffalo*

Corresponding author's email: prarany@buffalo.edu

A major focus in tissue engineering today is mimicking nature's pristine design with a particular emphasis on embryonic development that serves as a roadmap for regenerative medicine. The spatiotemporal presentation of morphogens, both soluble (biomolecules) and insoluble (matrix) serve as first principles for tissue engineering. Our research has focused on two discrete and synergistic fields, biomaterials and biophotonics. First, we utilize biomaterial systems to provide engineered niches for directed differentiation of stem cells. This encompasses defining topological biomaterial interfaces (insoluble, varying spatial scales) using electrospinning, gas-foaming and 3D printing as well as polymeric controlled release systems with agonist-antagonist combinations to generate synthetic morphogen fields. Second, we have investigated the ability of light-activated biological pathways in driving therapeutic biological responses. This use of non-thermal, non-surgical low dose biophotonics treatments is termed Photobiomodulation (PBM) Therapy. The ability to utilize PBM treatments as a non-invasive, easily customizable, and potent biophysical therapy has been noted to target fundamental disease pathophysiological responses such as pain, inflammation, immune responses, and tissue healing and regeneration. This has enabled a significant breadth of PBM clinical applications from concussion, Alzheimer's to wounds and mucositis among many others. Our work has demonstrated key roles for several photoactivated signaling pathways such as TGF-β1, NFκB, and ATF-4. These biological insights are being utilized to develop safe and effective clinical translational protocols for reproducible PBM clinical therapeutic outcomes. Finally, this talk focuses on brief overviews of our recent attempts at combing these two synergistic approaches to direct biological response for therapeutic clinical outcomes.

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Abstract **#2040**

BACTERIA-BASED MATERIALS FOR STEM CELL ENGINEERING

Manuel Salmeron-Sanchez[P][1]

[1] *University of Glasgow*

Corresponding author's email: manuel.salmeron-sanchez@glasgow.ac.uk

A new generation of materials has been developed over the last few years with highly dynamic properties that recapitulate properties of the extracellular matrix. These materials are not only highly dynamic but also very responsive to external stimuli to modulate their biophysical properties and biological functionalities, including sustained delivery of proteins upon external stimuli, mechanical properties that change as a function of time and self-healing capacity. These living materials are based either on advanced chemical functionalities (e.g. self-assembling peptides) or, alternatively, through the radical approach of using living cells that have been genetically modified and incorporated as living parts of the material. Particularly exciting are advances in using genetically modified microorganisms to achieve high functionalities, such as light-triggerable expression of adhesive peptides secretion of growth factors.

In this talk, we present bacteria-based materials as a new concept that supports stem cell engineering. We demonstrate that non-pathogenic bacteria such as Lactococcus Lactis can be engineered to expresses membrane proteins that present adhesion domains for mammalian cells (e.g. a fibronectin fragment that contains the integrin binding region) and also secrete growth factors and cytokines in a temporally regulated manner triggered by external stimuli. We present examples of bacteria-based materials that control the differentiation of mesenchymal stem cells towards osteogenic lineages both in 2D and 3D and also new bacteria-based materials that contribute to create a dynamic bone-marrow niche like environment that support the growth of mesenchymal stem cells.

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Abstract **#2041**

SILK FIBROIN BIOINKS FOR DIGITAL LIGHT PROCESSING (DLP) 3D BIOPRINTING

Chanhum Park[P][1,2]

[1] *Hallym University*

[2] *Nano bio regenerative medical institute*

Corresponding author's email*:* hlpch@paran.com

Three-dimensional (3D) bioprinting has been a highly influential technology in the field of tissue engineering to enable speedy and precise spatial patterning of cells, growth factors, and biomaterials. Bioink is one of the main factors in 3D bioprinting, and hydrogels are excellent matrix type by means of bioinks for 3D bioprinting. Recently, stereolithographic bioprinting via digital light processing (DLP) that allows high spatial resolution and rapid printing time of complex structures have attracted many studies. However, a small number of bioinks have been applied to DLP bioprinting in comparison with bioinks for other bioprinters. We developed a novel bioink based on silk fibroin that has been extensively used in biomedical fields due to its positive biological and biochemical properties as biomaterials. In this chapter, we summarized the silk fibroin basics and various applications of silk fibroin as a printing material. Also, the fabrication and performance of silk-based bioink for DLP bioprinter were discussed.

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Abstract **#2042**

ELECTRICAL APPROACHES FOR TISSUE REGENERATION

Sarah Cartmell$_P$[1]

[1] *The University of Manchester*

Corresponding author's email: sarah.cartmell@manchester.ac.uk

In the Cartmell laboratory, we have been developing different in vitro bioreactors that can apply different electrical regimes to cells and tissues with a view to influence cell activity: proliferation, differentiation and extracellular matrix production. This presentation will discuss the background evidence for using electrical regimes to influence cell and tissue activity as well as present our data on its use for bone, neural and skin regeneration.

Our research approach has also allowed us to further study the mechanism by which the activity of primary human mesenchymal stem cells are altered both in terms of cell proliferation and differentiation. A novel finding of the importance of the faradic by product of H2O2 proximal to the cathode as result of the direct electrical stimulus will be presented and its subsequent role in influencing primary mesenchymal stem cell proliferation. The morphology of primary cilia on these cells after electrical stimulation has been applied will also be discussed, in addition to the effect of varying electrical regime on cell response. The use of conducting polymers and piezoelectric materials to apply electrical regimes to the cells will be discussed.

This presentation will also present data on influence of electricity for chronic wound healing and on peripheral nerve repair.

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Kasama Srirussamee, Sahba Mobini2, Nigel J Cassidy, Sarah H Cartmell "Direct electrical stimulation enhances osteogenesis by inducing Bmp2 and Spp1 expressions from macrophages and pre-osteoblasts" Biotechnology & Bioengineering 2019 116 (12), 3421-3432

Abstract **#2043**

VOLUMETRIC BIOPRINTING

Christophe Moser[P][1], Damien Loterie[2], Paul Delrot[2], Jos Malda[3], Paulina Nunez Bernal[3], Riccardo Levato[3]

[1] *Ecole Polytechnique Federale de Lausanne*

[2] *Readily3D*

[3] *Utrecht University*

Corresponding author's email: christophe.moser@epfl.ch

Additive Manufacturing (AM) consists of a multitude of different technologies, offering different materials, with different quality outputs. However, all current AM technologies share the same major limitation: a three-dimensional object is fabricated layer after layer. The layered approach greatly limits the 3D design freedom and productivity in AM. It is no different in bioprinting where the tools to print cells are all based on a layered approach: extrusion, inkjet, laser induced forward transfer (LIFT) and Stereolithography.We pioneered a novel approach in bioprinting to overcome this layered approach to directly create objects in their 3D shape that we call volumetric printing by reverse tomography [1,2,3]. The method consists of sending dynamic light patterns from multiple angles corresponding to the projection of the 3D objects. The cell loaded photosensitive hydrogel accumulates the 3D distributed light dose and above a certain dose threshold, the hydrogel solidifies and forms a 3D scaffold. We have demonstrated the fabrication of a variety of complex 3D shapes with no restrictions on 3D design and with materials whose properties range from extremely soft (hydrogels) to hard (polymer acrylics) [1,3].Because this technique is based on 3D light dose accumulation, it is well suited for a control feedback loop, whereby the 3D dose is adjusted while the part is forming. We call this “intelligent” as it is controlling the manufacturing of the 3D object as it is built. We show the implementation of such a feedback loop and demonstrate experimentally a substantial gain in part fidelity.

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Abstract **#2044**

INTEGRATED HUMAN MULTI-ORGAN PLATFORMS FOR MODELING SYSTEMIC PATHOLOGIES

Gordana Vunjak-Novakovic[P][1]

[1] *Columbia University*

Corresponding author's email: gv2131@columbia.edu

Bioengineered “organs on a chip” platforms are evolving into a new paradigm for modeling human pathophysiology, with utility in both biological and preclinical studies. Establishing physiological communication between different tissues while preserving their individual phenotypes is a challenge that needs to be addressed to allow modeling of whole-body physiology in health and disease. An approach to meet these conflicting requirements is to establish a modular, configurable multi-organ platform in which each human tissue is cultured in its own optimized environment and linked to other tissues by vascular perfusion. We showed that under these conditions, the connected tissues (heart, liver, skin, bone) can maintain their molecular, structural and functional phenotypes over four weeks of culture. The platform allows individualized studies, as all tissues, endothelium and circulating cells can be derived from the same source of iPS cells. To illustrate the utility of this approach, we use two examples: (i) a simple liver-tumor platform that recapitulated clinical efficacy and toxicity for an anticancer drug, and (ii) a multi-organ platform that recapitulated the toxicity of doxorubicin observed in pediatric and adult clinical studies. Finally, we outline some of the opportunities and challenges for using these platforms in studies of development, physiology and disease.

Abstract **#2045**

HARNESSING THE EXTRACELLULAR MATRIX FOR DRIVING TISSUE REPAIR

Martin C. Harmsen[P][1]

[1] *University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology*

Corresponding author's email: m.c.harmsen@umcg.nl

Our body is equipped with the spatiotemporally tightly-regulated process of wound healing to cope with tissue damage in an organ-specific fashion. Dysregulated wound healing causes scarring and fibrosis in which excessive extracellular matrix (ECM) is deposited which usually causes organ failure. Given the dynamic and versatile nature of ECM and its instrumental role in tissue generation and regeneration, the relevance to understand and apply ECM in a therapeutic context stands to reason.

In homeostasis, ECM comprises of fibrous and sheet-like proteins such as collagens that are embedded in a water-retaining gel of highly negatively charged polysaccharides such as glycosaminoglycans. Essentially these 'reinforced hydrogels' instruct tissue cells, via adhesion motives and bound growth factors. We argued that isolated organ-specific ECM, formulated as

hydrogels and charged with therapeutic factors or their producing cells such as MSCs, would replicate nature's own use of ECM in tissue repair and regeneration.

Indeed adipose tissue-derived and cardiac left ventricular-derived ECM hydrogels not only efficiently bound the factors in the secretome of adipose tissue-derived stromal cells (ASCs) but also released these in a time-dependent sustained fashion. Cardiac hydrogels loaded with ASCs alleviated severe oncostatic drug-induced cardiomyopathy. Moreover, dermal-derived ECM hydrogels contributed to dermal healing in rats. In vitro, cardiac and skin-derived ECM hydrogels supported and stabilized formation of long-term vascular endothelial networks without the need of accessory cells such as pericytes. While virtually all tissue regeneration processes rely on adequate perfusion the application of pre-vascularized organ-specific ECM hydrogels offer an opportunity to augment healing.

Abstract **#2046**

TRANSLATIONAL STRATEGIES FOR SCALE UP EXPANSION OF CLINICAL-GRADE CELLS FOR TISSUE ENGINEERING APPLICATIONS

Joaquim Vives$_P$[1,2,3]

[1] *Banc de Sang i Teixits*

[2] *Vall d'Hebron Institute of Research*

[3] *Universitat Autònoma de Barcelona*

Corresponding author's email: jvives@bst.cat

Increasing demand of clinical-grade cells is a great challenge to developers and to the cell and gene therapy industry that will eventually industrialize production. This could be seen as an opportunity to evaluate existing bioprocessing technologies already tested in the production of vaccines and biologicals with great success. Herein we analyze the transition from manual cell expansion 2D methodologies to scalable and automated volumetric production platforms with the capacity to yield large numbers of cells per batch within established specifications in a robust, reproducible and cost-effective manner in compliance with current Good Manufacturing Practices. Moreover, Quality and Regulatory compliance must be addressed from bioprocess design and implementation to routine work. This presentation will review current trends and challenges of different manufacturing platforms, paying special attention to those supporting expansion of multipotent mesenchymal stromal cells in single-use bioreactors for further use in tissue engineering applications.

Keywords

bioreactor; mesenchymal stromal cell; translational medicine

Abstract **#2047**

B2B: A FIRST-OF-A-KIND 3D MODEL OF SPONTANEOUS BREAST CANCER METASTASIS TO THE BONE

Silvia Scaglione[P][1], Nicola Aceto[2,3], Andrea Banfi[4], Eric Farrell[5], Maurizio Aiello[6], Laura Martinelli[7], Jayden Smith[8], George Loudos[9], Lorenzo Moroni[10]

[1] *CNR*

[2] *University of Basel*

[3] *ETH Zurich*

[4] *USB, Basel*

[5] *Erasmus Medical Center, Rotterdam*

[6] *React4life s.r.l.*

[7] *IN s.r.l.*

[8] *CITC*

[9] *Bioemtech*

[10] *Maastricht university*

Corresponding author's email: silvia.scaglione@cnr.it

Recent advances in fluidic systems and 3D printing help to push the limits of in vitro models by adding a new layer of complexity: the connection between multiple organs. During the proposed symposium we aim to explore the most innovative and challenging aspects of the connection between multiple organoids and tissues by fluidic networks, spanning from the macro to micro integration in the vascular network to the propagation of vascularized organoids at clinically relevant dimensions. We will present the first-of-a-kind 3D model of spontaneous breast cancer metastasis to the bone and in particular the technological platform featuring two connected organoids in clinically relevant dimensions (cm3) and with an extensive network of capillaries that penetrate the whole mass. The connecting network between the organoids mimics the physiological blood circulation, with self-assembled micro-sized capillaries merging with bioprinted macro-sized vessels. Such in vitro platform recapitulates physiological tissue-level complexity with organoids comprising several million cells and therefore constitutes a more clinically relevant environment for in-depth studies.

B2B FET-OPEN project has selected the metastatic process of breast cancer to the bone as its first application and aims to provide a breakthrough technology to investigate metastasis longitudinally and at the single cell level. Therefore, in the B2B device a patient-derived breast cancer lesion is connected to an in vitro reconstructed bone, a marrow-containing ossicle. We will also highlight the B2B potential applications to study mechanism that rely on the inter-organ crosstalk.

Abstract **#2048**

UNRAVELLING THE MECHANICS OF MACROPHAGE-DRIVEN IN SITU TISSUE ENGINEERING IN HEALTH AND DISEASE

Anthal Smits$_P$[1,2]

[1] *Department of Biomedical Engineering, Eindhoven University of Technology*

2 Institute for Complex Molecular Systems, Eindhoven University of Technology

Corresponding author's email: a.i.p.m.smits@tue.nl

The use of acellular resorbable synthetic scaffolds for replacing diseased cardiovascular tissues, such as heart valves and blood vessels, is an attractive strategy that has shown great promise in recent preclinical studies and ongoing clinical trials. These scaffolds are designed to instantaneously take over the functionality of the replaced tissue upon implantation, and maintain functionality while they are gradually resorbed and replaced by autologous new tissue by infiltrating cells, directly in situ [1]. The interdependent processes of scaffold degradation and tissue formation are hypothesized to be orchestrated by macrophages, in cross-talk with myofibroblasts. However, our lack of fundamental insight into macrophage-driven regeneration under the influence of environmental factors, such as hemodynamic loads and patient-specific conditions, has led to unpredictable variability in outcome. In this presentation, I will share our recent efforts in the understanding and controlling of the in situ formation of functional new cardiovascular tissues (i.e. blood vessels and heart valves) by modulating the host immune response using resorbable supramolecular elastomers. Specifically, I will elaborate on our recent results regarding the influence of the local biomechanical loads, as well as the influence of patient-specific comorbidities, on the inflammatory and regenerative processes in response to such scaffolds, and how these may dictate tissue formation and remodelling.

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Abstract **#2049**

UNDERSTANDING CELLS-MATERIALS INTERACTIONS TO PROMOTE VASCULARIZATION

Elisabeth Engel[P][1,2]

[1] *Institute for Bioengineering of Catalonia*

2 Technical University of Catalonia (UPC)

Corresponding author's email: eengel@ibecbarcelona.eu

Efficient vascularization of tissue engineered grafts is still nowadays one of the main drawbacks for successful medical applications. Several strategies have been explored to promote angiogenesis and vascularization on tissue engineering scaffolds. From stem and progenitor cells, to growth factors or other biomolecules. Thus, ions have also been widely studied to activate residing cells to induce new vessels formation. However, the mechanisms involved in this process are not well understood. We have been exploring biomaterials that can activate angiogenesis in situ. The fabrication of scaffolds that can recreate instructive environments will send the specific signals to cells to produce a fast and equilibrated vascularization. In this talk I would review the effect of degradation products from the scaffolds on angiogenesis and will explore different models to evaluate this effect.

Abstract **#2050**

DEVELOPMENT OF A HUMAN BONE MARROW BIOMIMICRY

Sakis Mantalaris[P][1]

[1] *Georgia Institute of Technology*

Corresponding author's email: sakis.mantalaris@gatech.edu

Cellular microenvironments provide stimuli including paracrine and autocrine growth factors and physico-chemical cues, which support efficient in vivo cell production unmatched by current in vitro biomanufacturing platforms. While three-dimensional (3D) culture systems aim to recapitulate niche architecture and function of the target tissue/organ, they are limited in accessing spatiotemporal information to evaluate and optimize in situ cell/tissue process development. Herein, we present a human bone marrow biomimicry, which was optimized using a mathematical modelling framework that was parameterized by single-cell phenotypic imaging and multiplexed biochemical assays to simulate the non-uniform tissue distribution of nutrients/metabolites and growth factors in cell niche environments. The model was applied to a bone marrow mimicry 3D perfusion bioreactor containing dense stromal and hematopoietic tissue with limited red blood cell (RBC) egress. The model characterized an imbalance between endogenous cytokine production and nutrient starvation within the microenvironmental niches, and recommended increased cell inoculum density and enhanced medium exchange, guiding the development of a miniaturized prototype bioreactor. The second-generation prototype improved the distribution of nutrients and growth factors and supported a 50-fold increase in RBC production efficiency. This image-informed bioprocess modelling framework leverages spatiotemporal niche information to enhance biochemical factor utilization and improve cell manufacturing in 3D systems.

Keywords

Bioreactor; Modelling; Three-Dimensional

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Abstract **#2051**

NOVEL BIODEGRADABLE MATERIALS AND BIOINKS FOR THE BIOPRINTING AND REGENERATION OF DIFFERENT TISSUES

Rui L. Reis[P][1]

[1] *3B´s Research Group - Biomaterials, Biodegradables and Biomimetics, I3Bs, University of Minho, Guimarães, Portugal*

Corresponding author's email: ruilgreis@gmail.com

The selection of a proper material to be used as a scaffold or as a bioink in 3D bioprinting approaches in order to support or encapsulate cells is both a critical and a difficult choice that will determine the success of failure of any tissue engineering and regenerative medicine (TERM) strategy.

In our research group we have been using natural origin polymers, including a wide range of marine origin materials, for many different approaches that allow for the regeneration of different tissues. Several innovative bioinks with quite specific properties were proposed. We have also been optimizing the respective formulations for using these novel materials in distinct biomanufacturing strategies. This will be presented and discussed during the keynote talk.

Furthermore, an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation methodologies should be developed and optimized. We have been using different human cell sources namely: mesenchymal stem cells from bone marrow, mesenchymal stem cells from human adipose tissue, human cells from amniotic fluids and membranes and cells obtained from human umbilical cords.

The potential of each combination materials/cells, as related to different manufacturing technologies, with details focusing on bioprinting, to be used to develop novel useful regeneration therapies will be discussed. The use of different cells and new ways to assess their interactions with different natural origin degradable scaffolds and bioinks will be described. Several examples of TERM strategies to regenerate different types of tissues will be presented.

Abstract **#2052**

INDUCTION OF TISSUE REPAIR BY ACTIVATION OF ENDOGENOUS STEM / PROGENITOR CELLS

Ranieri Cancedda[P][1]

[1] *University of Genova, Italy*

Corresponding author's email: ranieri.cancedda@unige.it

For repair of chronic or difficult to heal tissue deficits, major constraints exist to a broad application of cell therapy and tissue engineering approaches, i.e. transplantation of “ex vivo” expanded autologous stem/progenitor cells, alone or associated to carrier biomaterials. In order to enable a large number of patients to benefit from a Regenerative Medicine approach, new strategies and new products should be considered. To develop innovative regenerative therapies, we should learn from Mother Nature. In all injured tissues - apart few tissues such as hyaline cartilage - there is a common initial stage after the damage, that is characterized by hematoma, clot formation and platelet activation. Platelets, activated by the injured tissue contact, release a variety of growth factors and bioactive molecules via a degranulation process. These factors and molecules play a significant role in triggering the healing process and are crucial for regulating immune cell migration and the creation of an inflammatory microenvironment at the site of the wound. Following this first inflammatory phase, repair or regeneration of the tissue may occur through paracrine signals activating the revascularization of the wound and recruiting or turning on at the wound site cells with healing potential, such as stem cells, progenitors or undifferentiated cells derived from tissue differentiated cells. Given the triggering role played by platelets in the tissue healing process, we developed new platelet derived products and we propose their local application to treat chronic or acute, often severe, damages in different organ districts.

Abstract **#2053**

DUAL CROSS-LINK HYDROGELS WITH TUNABLE VISCOELASTICITY CONTROL STEM CELL DIFFERENTIATION.

Chiara Pizzolitto$_P$[1]

[1] *University of Trieste*

Corresponding author's email: psacco@units.it

Mechanotransduction recapitulates the conversion of external mechanical information into intracellular biochemical response. To this, it was recently recognized that stem cell fate can be markedly influenced by surrounding extracellular matrix (ECM) mechanics. Most of our knowledge in cell mechanobiology has been built using purely elastic materials as model of ECM. However, native tissues do not exhibit purely elastic response but manifest viscoelasticity, that is a time- and frequency-dependence to loading. In fact, recent works have proved that stress-relaxation or plasticity, and more broadly, viscosity-driven processes can be considered as potent modulators of stem cell behavior. To recapitulate native tissue mechanics and provide a more realistic ECM model, here we present unprecedented viscoelastic substrates showing adaptable viscoelasticity. In particular, I will present an innovative dual cross-link gel system based on a chitosan derivative, shortly named CTL, assembled via both temporary and permanent cross-linkers.[1] Concepts related to macromolecular chemistry and physics will be disclosed. Of note, temporary junctions are exploited to finely tune material viscoelasticity. I will show how resulting hydrogels result optimal substrates for cell anchoring upon coating with ECM proteins. Though endowed with similar elasticity, a viscosity-cell function relationship will be unveiled, identifying high viscosity hydrogels as superior materials in fostering stem cell differentiation toward a bone-like phenotype with respect to more elastic counterparts. Taken together, these results lay the groundwork for additional investigations on the role played by substrate viscoelasticity in directing cell-fate decisions.

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Abstract **#2054**

SUBSTRATE DISSIPATION ENERGY REGULATES CELL ADHESION AND SPREADING

Pasquale Sacco[1], Chiara Pizzolitto[1]

[1] *University of Trieste*

Corresponding author's email: chiara.pizzolitto@phd.units.it

Recent evidences have led to hypothesize that dissipation of energy through viscoelastic extracellular matrix (ECM) could play a cardinal role in directing cell-fate decisions, but whether and how it correlates with specific cell response has remained unclear up to date. In this talk I will introduce substrate dissipation energy as novel cell-fate controller.[1] Specifically, I will illustrate recent findings about viscoelastic and plastic chitosan-based substrates endowed with different dissipative energies capable of modulating cell behavior in terms of adhesion and spreading. While keeping constant stress relaxation and systematically decoupling overall stiffness from linear elongation, we have introduced an energy dissipation term (J/mol), that is the molar energy required to deviate from linear stress-strain regime and enter into plastic region. Strikingly, we have unveiled an inverse relationship between substrate energy dissipation and cell response, with high adhesion/high spreading and low adhesion/no spreading detected for substrates at low and high dissipation energy, respectively. We concluded that cells decide how to react depending on the effective energy they can earmark for their functions. Of note, I will show how combinations of facing 5-consecutive sugars (pentads) composing substrates are essential in damping shear stress, thus behaving as cell traction forces dampers. Collectively, in this talk I will illustrate how the crosstalk between cells and ECM can be considered as energetic in origin.

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Abstract **#2055**

INTRODUCING THE COMMERCIALIZATION OF 4D PRINTING: CONTROLLING MATERIALS INSIDE THE BODY FROM THE OUTSIDE

Thomas Webster[P][1]

[1] *Northeastern University*

Corresponding author's email: websterthomas02@gmail.com

The presentation will introduce the commercialization of 4D printing (with the 4th dimension representing time) where self-assembled materials are created and when stimulated by near infra red (NIR) on-demand, can change shape to improve stem cell delivery, drug delivery, increase localized pressure to promote tissue growth, and so much more. Such materials are being commercialized for a wide range of medical applications including treating neurological diseases, pain addiction, and for regenerative medicine. This talk will cover both in vitro and in vivo studies. Moreover, it will emphasize how such materials can be used to minimize the disruption of stem cells in culture for in vivo delivery.

Abstract **#2056**

SPATIALLY NANOPATTERNED SCAFFOLDS PROMOTE THE SURVIVAL OF INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS IN THE ISCHEMIC LIMB

Ngan Huang[P][1], Guang Yang[1], Cynthia Alcazar[2], Caroline Hu[2], Tatiana Zaitseva[3], Michael Paukshto[3]

[1] *Stanford University*

[2] *Veterans Affairs Palo Alto Health Care system*

[3] *Fibralign Corporation*

Corresponding author's email: ngantina@stanford.edu

Introduction: Dysfunctional endothelial cells (EC) can restrict flow in the extremities, leading to limb ischemia. We aim to improving revascularization of ischemic limbs using cell therapy, augmented with spatially patterned scaffolds that promote survival. The purpose was to evaluate the effect spatially patterned scaffolds on endothelial cell survival. Methods: The biophysical parameters of collagen scaffolds (alignment, crosslinking, and fibril diameter) were characterized with atomic force microscopy and degradation assays. Cytoskeletal arrangement of primary human coronary artery ECs and human induced pluripotent stem cell (iPSC)-derived ECs on collagen scaffolds was evaluated by F-actin staining. To evaluate the effect of mechanical properties on cell viability in vitro, Live/Dead and MTS assays were used. Cell seeded scaffolds were implanted into a mouse hindlimb ischemia model, and cell survival was tracked by bioluminescent imaging. Results: Both primary EC and iPSC-ECs reorganized their F-actin cytoskeleton corresponding to the alignment of the fibrils, irrespective of crosslinking degree, stiffness or fibril diameter. When cells were grown in hypoxic mimicking ischemia however, viability was improved using aligned scaffolds with 200 nm fibril diameter. Degradation assays showed that scaffolds with higher crosslinking had significantly more durability. When cell-seeded scaffolds were implanted to the ischemic limb, the aligned scaffolds with 200nm fibril diameter had the highest survival, confirming in vitro studies. Conclusions: Aligned collagen scaffolds with 200nm fibril diameter and high cross-linking degree led to improved viability of primary and iPSC-ECs in a mouse model of PAD.

Abstract **#2057**

ENGINEERED BIOMIMETIC HYDROGELS WITH COMBINATORIAL CELL ADHESIVE LIGANDS FOR ANGIOGENESIS

Ngan Huang[P][1], Mahdis Shayan[1], Riley Suhar[1], Sarah C Heilshorn[1]

[1] *Stanford University*

Corresponding author's email: ngantina@stanford.edu

Vascular endothelial cells are a promising cell type for treating ischemic cardiovascular tissues. However, the low survival of transplanted cells is a bottleneck of stem cell therapy. To enhance cell survival and function, we developed a new family of engineered ECMs with independently tunable cell-adhesive biochemical domains. Elastin-like proteins (ELPs) are genetically engineered proteins that promote cell adhesion through customizable amino acid sequences for cell adhesion and provides elasticity and structural support through its elastin-like domain, derived from the elastin VPGXG sequence. ELPs with adhesive ligands (RGD, YIGSR, PLAEIDGIELTY) and non-adhesive ligands (RDG) were developed. We investigated the effect of three cell adhesive ligands (RGD, YIGSR, PLAEIDGIELTY) on endothelial cell survival using. To control the density of adhesive ligands, ELPs containing adhesive ligands were mixed with RDG-ELP at different ratios while maintaining the constant 10mg/ml ELP concentration. We used a systematic Design of Experiment (DOE) approach to determine the individual and combinatorial effects of three ligand concentrations for the survival and function of endothelial cells. Human microvascular endothelial cells were seeded onto ELP arrays and their survival and phenotype were analyzed under conditions of hypoxia (1% O2) and reduced nutrients (1% fetal bovine serum), as is present in ischemic injury sites. We identified combinatorial ELP-RGD and ELP- PLAEIDGIELTY improves cell survival and CD31 phenotype expression in comparison to YIGSR-ELP and individual ELPs. These results suggest that optimal combinatorial ELPs improve the survival and function of endothelial cells under hypoxia conditions and hold great promise for cell delivery for ischemic tissues.

Abstract **#2058**

EXTRACELLULAR MATRIX CUES MODULATE ENDOTHELIAL-TO-MESENCHYMAL TRANSITION

Ngan Huang$_P$[1], Maedeh Zamani[1]

[1] *Stanford University*

Corresponding author's email: ngantina@stanford.edu

Introduction: Recent studies demonstrate the occurrence of endothelial-to-mesenchymal transition (EndoMT) within atherosclerotic plaques. The aim of this study is to investigate the effect of mechanical properties of the underlying matrix on progression of EndoMT under inflammatory condition associated with atherosclerosis. We hypothesize that EndoMT is modulated by the stiffness of the underlying extracellular matrix. Materials and Methods: Human coronary artery endothelial cells (HCAECs) were cultured on fibronectin-modified polyacrylamide gels (4 kPa or 100 kPa) or tissue culture plastic (TC, 105 kPa) in endothelial basal media (Lonza) with/without exposure to the inducing agent, transforming growth factor-β (TGF-β (20ng/ml). qPCR and immunostaining of endothelial, mesenchymal, and EndoMT markers, were investigated at for up to 6 days.

Results and Discussion: Results demonstrated that at day 6, HCAECs expressed mesenchymal (α-SMA, SM22, Calponin) and EndoMT (SNAIL,TWIST) markers in a stiffness-dependent manner, where stiffer substrates induced more phenotypic transition. Interestingly, in the presence of TGF- β which is known to induce EndoMT, the hydrogels with physiological stiffness could partially abrogate the expression of mesenchymal markers. Immunofluorescence staining showed that increasing the substrate stiffness, SM22 and calponin protein expression was notably increased. Semi-quantitative analysis of SNAIL expression demonstrated that SNAIL expression was mainly localized in cytoplasm of HCAECs seeded on hydrogels, compared to the cells seeded on TC with higher levels of nuclear expression. Conclusions: Substrate stiffness mediates EndoMT and should be considered in treating atherosclerosis.

Abstract **#2059**

SPATIALLY PATTERNED SCAFFOLDS DRIVE ORGANIZED VASCULAR REGENERATION

Ngan Huang$_P$[1], Karina Nakayama[2], Cynthia Alcazar[3], Caroline Hu[3], Thomas Rando[1]

[1] *Stanford University*

[2] *Oregon Health Sciences University*

[3] *Veterans Affairs Palo Alto Health Care System*

Corresponding author's email: ngantina@stanford.edu

Ischemic cardiovascular diseases such as peripheral arterial disease (PAD) can lead to severe occlusion of the blood vessels within the skeletal muscle in the extremities. A successful therapeutic intervention must not only restore tissue vasculature, but also the physiological anatomical structure of microvascular networks. Towards this goal, we developed spatially patterned scaffolds to promote the organized formation of aligned microvascular networks in vivo. Three-dimensional collagen scaffolds having parallel-aligned or randomly-oriented nanofibrils were fabricated and seeded with mouse myoblasts and human microvascular endothelial cells (ECs). The co-cultured cells within parallel-aligned nanofibrillar scaffolds produced endothelialized and aligned myotubes with highly synchronized muscle contractility, as well as cellular alignment along the direction of the parallel-aligned nanofibrils, compared to co-cultured cells on randomly-oriented scaffolds. When co-seeded with myoblasts in aligned scaffolds, ECs secreted significantly more angiogenic and/or myogenic cytokines, than when co-cultured in randomly oriented scaffolds. Transplantation of co-cultured cells on aligned scaffolds into ablated muscle resulted in the formation of parallel-aligned myofibers interspersed with parallel-aligned microvasculature networks, along with significantly greater vascular perfusion, compared to treatment of co-cultures on randomly-oriented scaffolds. This work demonstrates that endothelialized and aligned engineered skeletal muscle promotes organized microvascular network formation, and has therapeutic significance for the treatment of PAD.

Abstract **#2060**

INSULIN-LIKE GROWTH FACTOR-1 LADEN SCAFFOLDS WITH REHABILITATIVE EXERCISE IMPROVES MUSCLE REGENERATION

Ngan F Huang$_P$[1], Karina Nakayam[2], Cynthia Alcazar[3], Caroline Hu[3], Thomas Rando[1]

[1] *Stanford University*

[2] *Oregon Health Sciences University*

[3] *Veterans Affairs Palo Alto Health Care System*

Corresponding author's email: ngantina@stanford.edu

Muscle regeneration can be irreversibly impaired by traumatic injuries, despite the high regenerative capacity of skeletal muscle. The objective of this study is to modulate and enhance the regenerative process via myogenic growth factors and in conjunction with rehabilitative exercise for the treatment of volumetric muscle loss. To enhance the regenerative potential of injured skeletal muscle, insulin growth factor-like-1 (IGF-1)-laden nano-patterned anisotropic scaffolds were fabricated by an extrusion process, followed by overnight incubation with IGF-1. Individual scaffolds released a cumulative total of 1250 ng $\pm$ 150 ng of IGF-1 in vitro over the course of 21 days. When implanted into a murine injury model by ablation of the tibialis anterior muscle, the growth factor laden scaffolds in conjunction with voluntary caged wheel exercise could significantly improve the density of isolectin+/CD31+ perfused microvessels by greater than 3-fold in comparison to treatment of constructs without IGF-1. Enhanced myogenesis was also observed in the muscle treated with the IGF-1 laden scaffolds combined with exercise compared to the same IGF-1 laden scaffolds transplanted into mice that did not receive exercise. Furthermore, the abundance of neuromuscular junctions was greater when treated with IGF-1 laden scaffolds in conjunction with exercise, in comparison to the same treatment without exercise. These findings demonstrate that voluntary exercise improved the regenerative effect of growth factor-laden scaffolds by augmenting vascular regeneration and myogenesis, and has important translational implications in the therapeutic design of off-the-shelf therapeutics for the treatment of traumatic muscle injury.

Abstract **#2061**

A PRELIMINARY STUDY OF DECELLULARIZED WHOLE-ORGAN PRE-VASCULARIZATION: A NOVEL APPROACH FOR ORGANOGENESIS

Ibrahim Fathi[P][1,2], Takehiro Imura[1], Akiko Inagaki[1], Masafumi Goto[1]

[1] *Division of Transplantation and Regenerative Medicine, Graduate School of Medicine, Tohoku University, Japan*

[2] *Department of Surgery, School of Medicine, Alexandria University, Egypt*

Corresponding author's email: dr_vk169@hotmail.com

PURPOSE. To examine the suitability of pre-vascularized whole-liver ECM as a site for pancreatic islet transplantation.
METHODS. Whole-Livers from male Lewis rats were decellularized through arterial perfusion of detergents. The decellularized liver scaffold was implanted into Lewis rats and an arteriovenous bundle was passed through the scaffold. At the time of implantation, fresh bone marrow preparation (BM; n=3) or adipose-derived stem cells (ADSCs; n=4), or HBSS (n=4) was injected into the scaffold through the portal vein. After 5 weeks, around 2,600 IEQ of islets were injected through portal vein of the scaffold. The recipient rats were made diabetic by injection of 65 mg/kg STZ i.v., and were followed up by measuring blood glucose and body weight for 30 days. Intravenous glucose tolerance test was performed in the cured animals and samples were collected for immunohistochemical analysis (IHC). Micro-CT images were obtained from one rat in each group for representation.
RESULTS. Two rats in the BM group and one rat in the ADSCs group showed normalization of blood glucose, while one rat from each group showed partial correction of blood glucose. In contrast, no rats were cured in the HBSS group. Micro-CT showed evidence of sprouting from the arteriovenous bundle inside the scaffold. IHC for insulin, BrdU, and vWF is currently ongoing and the results will be provided.
CONCLUSION. Despite the limited numbers, the study showed the promising potential of the pre-vascularized whole-organ scaffold as a novel approach for islet transplantation. Both BM and ADSCs were superior to the acellular scaffold.

Keywords

Prevascularization; Axial vascularization; Decellularized liver

Abstract **#2062**

CHALLENGES AND OPPORTUNITIES IN CLINICAL TRANSLATION OF HYDROGELS

Jöns Hilborn$_P$[1]

[1] *Uppsala University*

Corresponding author's email: jons.hilborn@kemi.uu.se

We selected hyaluronic acid (HA) as suitable starting material for the design of tissue inductive hydrogel systems with potential for clinical translation. Key decisive factors included earlier demonstrated safety, the possibility of sterilization, non-immunogenicity, quality assurance, IPR and cost-effectiveness. HA was derivatized to permit surgically acceptable sterile handling with mixing, injection and suitable gel formation by click type hydrazide/hydrazone formation. Growth factor stability, cytotoxicity, genotoxicity and preclinical toxicity studies proceeded translation to bone regeneration and MSC based therapies. Interestingly, calcium phosphate filler in HA gels allow rapid angiogenesis and facile large bone formation even with limited amounts of growth factors which were not predictable from in-vitro evaluations. In general, extrapolation from in vitro data to in vivo responses is often inappropriate. Therefore, we employed an iterative strategy including early in vivo screening, e.g. multiple subcutaneous injections in the rat model allowing reference in the same animal, to adjust our system before large animal studies. We took utterly care of our pigs to assure low-stress levels as stress known to influence the outcome. Our learning is that reducing complexity is a must when facing the roadblocks of reality when moving towards translation. Also, HA-based hydrogels, and many other gels, have inherent limitations that include unwanted swelling in vivo and low mechanical strength. They are rarely strong enough to be attached to tissues with current surgical techniques or even to shape-holding prefabricated structures.

Abstract **#2064**

AI-MEETS CELL MORPHOLOGY: A NEW BIOLOGICAL BIG DATA FOR CELL EVALUATION

Ryuji Kato[P][1]

[1] *Graduate School of Pharmaceutical Sciences, Nagoya University*

Corresponding author's email: kato-r@ps.nagoya-u.ac.jp

Morphology of cells has been important and trustworthy information to monitor cellular status non-invasively. From the beginning of cell culture history till now, such cellular morphological information is one of the most practically used techniques in cell culture, and it is still an essential skill for cell biologists to control cellular quality. However, such morphological information had long been evaluated subjectively by the intentionally measured parameters or by champion image selected from the culture vessel.

However, by the advances of hardware technology that enable automatic and comprehensive imaging, combined with other rapid growing technologies in artificial intelligence (AI)-related technologies, such image-derived morphological information is now becoming to be expected as a new biomarker to non-invasively evaluate the cells quantitatively.

Our group has long been reporting various applications of such AI-guided morphological cell evaluation methods for cellular quality evaluation [1,2]. This technique is now strongly expected as one of the most effective tools to monitor cellular quality during the massive cell manufacturing in cell therapies.

We here summarize the application possibilities and its technological advancements in such AI-guided morphological cell evaluation technology and introduces its important applicability in understanding the scaffold design for more effective tissue engineering.

Acknowledgments

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Abstract **#2065**

MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) IN ENHANCING MACROPHAGE AND MONOCYTE CELL ADHESION: POTENTIAL VASCULAR RE-ENDOTHELIALISATION APPROACH

Rohaina Che Man[P][1], Muhammad Dain Yazid[2], Mohamad Fikeri Ishak[2], Mohd Ramzisham Abdul Rahman[3], Raja Zahratul Azma Raja Sabudin[1], Nadiah Sulaiman[2]

[1] *Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia*

[2] *Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia*

[3] *Department of Surgery, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia*

Corresponding author's email: rohaina@ppukm.ukm.edu.my

Monocyte chemoattractant protein-1 (MCP-1) is one of the pro-inflammatory chemokine that is positively expressed in atherosclerotic plaque and in the intimal area during arterial injury. Selection of the suitable chemokines with the combination of cytokines may have a promising effect on halting the progression of intimal hyperplasia. The objective of this study was to determine the effect of MCP-1 in enhancing macrophage and monocyte cell adhesion. 10ml of venous blood was acquired from adult healthy volunteers after obtaining written consent. PBMCs isolation was performed within 1 hour of blood collection using SepMate tubes. Isolated PBMCs were resuspended in the endothelial cells growth medium and seeded onto 48-well plate, that were firstly coated with either 1ng/ml, 20ng/ml or 50 ng/ml of MCP-1. The cells with a seeding density of 1.2 × 105 cells per well were incubated for 90 minutes at 37°C. Non-adherent cells were removed and attached cells were fixed and mounted. Photomicrographs of the adherent cells were captured and quantified manually in ten representative fields per well. After 90 minutes of incubation, the cultured cells in the plate coated with 50ng/ml of MCP-1 showed significantly higher cell attachment (32794.12 ± 19007.28 cell/cm2) compared to the cells cultured on 20ng/ml (25500 ± 10697.96 cell/cm2), 1ng/ml (21156.86 ± 7833.85 cell/cm2) of MCP-1 and control group without coating (11617.65 ± 3045.24 cell/cm2), respectively. Hence, we concluded that MCP-1 chemokines coating provides the suitable niche environment in enhancing macrophage and monocyte cell adhesion, which could potentially be used for future vascular re-endothelialisation.

Abstract **#2066**

TISSUE-ENGINEERING THE UPPER RESPIRATORY TRACT

Nick Hamilton[P][1]

[1] *University College London*

Corresponding author's email: nick.hamilton@ucl.ac.uk

Cases of human bioengineered circumferential tracheal implants have been complicated by poor mucosalization following implantation resulting in infection, mucostasis and airway obstruction. We investigated methods of regenerating a respiratory epithelial graft that could be used as part of a bioengineered transplant to replace a damaged section of the upper airway.

The optimal extracellular matrix environment for respiratory epithelial cell attachment and proliferation was examined. Collagen IV was shown to be the leading protein mediating epithelial attachment acting via the integrin α2β1 and a Src and FAK mediated intracellular pathway. Laminin played a key role in respiratory epithelial cell proliferation and was dependent on an integrin pathway containing the subunit β1.

Biological, synthetic and biomimetic scaffolds were compared for their suitability to sustain and differentiate a respiratory epithelial layer. A human dermis scaffold was found to preserve a collagen IV and laminin layer following decellularization and was used to successfully regenerate a full thickness human respiratory epithelial layer containing mucus secreting goblet cells and motile ciliated epithelium.

The regenerated layer was implanted onto a section of pre-vascularized decellularized trachea in-vivo, although differentiation markers were lost following one week of implantation. This demonstrated the limitations of implanting full-thickness layers and led to an alternative strategy utilizing respiratory basal cells protected in a dehydrated collagen matrix. Trials using this method successfully established a long-term epithelial layer on decellularized trachea in-vivo.

This approach provides an effective method for re-epithelizing hollow-organs and overcomes the key rate-limiting step of an inadequate epithelial barrier following implantation.

Abstract **#2067**

OPTIMISATION OF FRACTALKINE (CX3CL1) CONCENTRATION IN MONOCYTE/MACROPHAGE RECRUITMENT AND ADHESION TO PROMOTE VASCULAR RE-ENDOTHELISATION.

Mohamad Fikeri Ishakp[1], Rohaina Che Man[2], Muhammad Dain Yazid[1], Raja Zahratul Azma Raja Sabudin[2], Mohd Ramzisham Abdul Rahman[3], Nadiah Sulaiman[1]

[1] Centre for Tissue Engineering & Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia

[2] Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia

[3] Department of Surgery, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia

Corresponding author's email: mfi@ppukm.ukm.edu.my

Fractalkine (CX3CL1) is a type of chemokines that plays a substantial role in inflammatory vascular disorders. Cytokine and chemokine have the potential to be utilised as recruitment factors to target inflammatory related diseases. Thus, this study aims to evaluate the effect of different concentration of CX3CL1 in promoting macrophage and monocyte adhesion. 10ml of venous blood collected with consent from healthy subjects. Peripheral blood mononuclear cells (PBMCs) was isolated using SepMate tubes and resuspended in endothelial cells growth medium. PBMCs were then seeded onto 48-well plate that was pre-coated with 1ng/ml, 20ng/ml and 50 ng/ml of CX3CL1. The cells with a seeding density of 1.2 × 105 cells per well were incubated for 90 minutes at 37°C. Non-adherent cells were removed and attached cells were fixed and mounted. Photomicrographs of the adherent cells were captured and quantified manually in ten representative high-powered image fields (40X magnification) per well. Cell quantification shows that plate coated with 1ng/ml of CX3CL1 exhibit higher monocyte/macrophage attachment (28764.71 ± 1373.66 cell/cm2) as compared to plates coated with 20ng/ml (24823.52 ± 15983.59 cell/cm2) and 50ng/ml (20941.17 ± 12886.83 cell/cm2) of CX3CL1. The control group without coating display the lowest cell yield (16470.58 ± 645.27 cell/cm2). As a conclusion, this preliminary data suggests that CX3CL1 promotes macrophage and monocyte adhesion at a minute concentration of 1ng/ml and has the potential to be applied in vascular graft re-endothelisation and repair.

Abstract **#2068**

MICROENVIRONMENT BY MACROMOLECULE: HOW CROWDING BOOSTS MSC DIFFERENTIATION AND PHYSIOLOGY

Michael Raghunath[P][1,2,3,4]

[1] *Center for Cell Biology and Tissue Engineering*

2 Institute of Chemisty and Biotechnology

3 Zurich University of Applied Sciences (ZHAW)

4 Wädenswil, Switzerland

Corresponding author's email: ragh@zhaw.ch

All cultured cells were in a context of other cells and a meshwork of extracellular matrix (ECM) that was richly decorated with growth factors, exosomes, and matricellular ligands. Abruptly removed from their former microenvironment, cells must survive in vitro on stiff plastic and hold onto adhesive proteins adsorbed from serum or coatings. As cells have co-evolved with ECM, they developed respective receptors, such as integrins. Obviously, these receptors did not evolve to recognise titanium alloys, thermoplastic polymers, or hydrogels. Therefore, these materials need to be endowed with peptide sequences that represent recognition signals for receptors mediating cell adhesion. In all the excitement about new components and shapes of biomaterials and their "functionalisation", the ability of the cells to make their own microenvironments has been widely neglected. Ironically, cells in culture can make ample ECM microenvironments, but our standard aqueous culture conditions do not let them. This can be remedied by recreating conditions of macromolecular crowding (MMC). Following up on John Bateman's work in the early 90ies, I have been developing MMC systematically for tissue engineering to empower cells to make their own microenvironments. Using MMC we have been able to create substantial ultraflat 3D matrix environments that greatly influence stem cell differentiation via dynamic cell-matrix reciprocity. MMC-treated monolayer cultures show cells embedded in ECM cocoons, reflected by surround expression of focal adhesions, even on top of cells. This mechanobiological feature, brought about by MMC, is able to unlock a brown differentiation repertoire in human bone marrow-derived stem cells.

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Abstract **#2069**

HUMAN iPSC-DERIVED CHONDROCYTES IN GELMA AS A PROMISING INK FOR CARTILAGE REGENERATION

Hannah Agten[P][1,2,3], Inge Van Hoven[2], Samuel Ribeiro Viseu[2], Jasper Van Hoorick[4], Sandra Van Vlierberghe[4], Frank P Luyten[1,2], Veerle Bloemen[1,3]

[1] Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, O&N 1, Herestraat 49, Box 813, 3000 Leuven, Belgium

[2] Skeletal Biology and Engineering Research Center, KU Leuven, O&N 1, Herestraat 49, Box 813, 3000, Leuven, Belgium

[3] Materials Technology TC, Campus Group T, KU Leuven, Andreas Vesaliusstraat 13, 3000 Leuven, Belgium

[4] Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Krijgslaan 281, S4-Bis, 9000 Ghent, Belgium

Corresponding author's email: hannah.agten@kuleuven.be

Joint surface defects in articulating joints have limited healing potential and can extend into the underlying subchondral bone. In most patients, osteochondral defects evolve into chronic osteoarthritic disease, when left untreated. Tissue engineering strategies therefore aim at treatment of joint surface defects in an early stage. One approach focuses on cell-based 3D constructs capable of regenerating damaged tissue. However, lacking process reproducibility and limiting design complexities have hampered translation into clinically relevant products. Recent advances in additive manufacturing have paved the way for extrusion-based bioprinting, an enabling technology that could overcome these limitations. In this study, methacrylated gelatin (gelMA) was combined with human iPSC-derived chondrocytes, as a bioink to produce cartilaginous tissue analogs. After crosslinking the bioink with Li-TPO-L photo-initiator (365nm) in polytetrafluorethene moulds, cell-laden gel constructs (4x4x2mm³) were cultured in chondrogenic medium for 28 days. Histology showed homogeneous cell distribution, with increasing amount of cells, as also confirmed by DNA quantification. The cell-laden constructs were Alcian Blue- and Safranin O-positive, showing sulphated glycosaminoglycan (GAG) deposition uniformly distributed throughout the construct. Pericellular collagen type II deposition throughout the constructs was shown with immunohistological stainings, whereas collagen type I was only present on the outer surface. Gene expression corroborated with histology, showing upregulation of ACAN, COL2A1 and COL1A1. Taken together, these data indicated that hiPSC-derived chondrocytes encapsulated in gelMA are able to maintain their phenotype and produce cartilage matrix in vitro. This suggests that the formulation can serve as a promising bioink for bioprinting strategies aiming to produce stable cartilage implants.

Keywords

Cartilage tissue engineering; iPSC-derived chondrocytes; gelMA

Abstract **#2070**

WHAT INDUSTRY NEEDS FROM RESEARCHERS FOR A SUCCESSFUL HARD/SOFT TISSUE ADHESIVE PRODUCT?

Jens Thies[P][1]

[1] *DSM Biomedical*

Corresponding author's email: jens.thies@dsm.com

Whilst there are many exciting and promising lines of biomaterials research that may lead to the breakthroughs of tomorrow, one of the greatest challenges today is how to commercially translate these successfully from lab to patient. The requirements for an evidence-based approach and the new MDR requirements make disruptive innovation in biomaterials harder today than ever.

One of the clinical dreams is a tissue adhesive that could enable clinicians to easily and safely repair a broad range of tissue types. There have been many candidates proposed over the last 50 years. These fall broadly into two groups: synthetic chemically engineered and bioinspired biomimetic adhesives. Example of the former are n-butyl cyanoacrylates and novel polyurethanes whilst the latter includes adhesives derived from biology such as fibrin glues and adhesive proteins from a range of animal and plant sources. The mechanisms of adhesion differ and some may fall into drug regulation and others may be considered devices.

The human body contains a broad range of tissue types and despite the advances in surgical techniques and materials there are many unmet clinical needs where an adhesive technology would augment or even replace current state of the art materials. Examples such as meniscal tear repair where sutures are the main attachment strategy and a safe and effective adhesive technology would transform each procedure.

Any new paradigm of treatment with an adhesive has to have a well thought out patient journey that clearly demonstrates the clinical benefit to patient, surgeon and ultimately the healthcare providers.

Abstract **#2071**

MECHANISTIC MATHEMATICAL MODELLING FOR REGENERATIVE MEDICINE

Sarah Waters[P][1]

[1] *Mathematical Institute, University of Oxford*

Corresponding author's email: waters@maths.ox.ac.uk

Successful clinical translation of regenerative medicine therapies requires robust preclinical tools for safety and efficacy. Typically, preclinical toolkits integrate in vitro and in vivo approaches. In this talk, I will showcase how mathematical modelling, in combination with in vitro and in vivo experimental approaches, can enhance the predictive capability of preclinical research and facilitate the optimisation of safe clinical cell therapies. We demonstrate the enormous potential for embedding in silico predictive mathematical modelling into preclinical toolkits to boost the clinical translation of preclinical research.We consider mathematical modelling of cell therapies. In cell therapies, stem cells are delivered to damaged tissues to promote their regeneration. We show how a mechanistic mathematical approach can model how cells reach the injury site, e.g. by labelling cells with magnetic particles and using externally applied magnetic fields to guide the cells to the target site; provide insights into the immune response; assess the degree of cell engraftment within the injury site; and determine how the cells can be modified, e.g., via encapsulation, to optimise their therapeutic role.

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Abstract **#2072**

REGENERATIVE INTERVENTIONS USING STEM CELLS SECRETOME IN INJURY AND DEGENERATION OF THE CENTRAL NERVOUS SYSTEM

Antonio J Salgado[P][1]

[1] *ICVS, School of Medicine, University of Minho, Braga, Portugal*

Corresponding author's email: asalgado@med.uminho.pt

The low regeneration potential of the central nervous system (CNS) represents a challenge for the development of new therapeutic strategies. Mesenchymal stem cells (MSCs) have been proposed as a possible therapeutic tool for CNS disorders, namely due to the beneficial actions of their secretome. Indeed, the latter possesses a broad range of neuroregulatory factors that promote an increase in neurogenesis, inhibition of apoptosis/glial scar, immunomodulation, angiogenesis, neuronal and glial cell survival, as well as relevant neuroprotective actions into different pathophysiological contexts. Considering their protective action in lesioned sites, MSCs, and their secretome, might also improve the integration of local progenitor cells in neuroregeneration processes. In this sense their use could represent an important vehicle for the establishment of future CNS regenerative therapies. In the present talk the role of MSCs, and their secretome, on phenomena such as in vitro and in vivo neuronal/glial survival will be addressed. Additionally, their possible applications, for Parkinson's Diseases and Spinal Cord Injury regenerative medicine will also be presented.

Abstract **#2073**

NOVEL LIPOSOMAL DRUG DELIVERY AND TARGETING APPROACHES FOR TISSUE REGENERATION

Sophia G Antimisiaris[P][1,2]

[1] *University of Patras, Greece*

[2] *FORTH/ICES, Greece*

Corresponding author's email: santimis@upatras.gr

The application of liposomal nanotechnology for the development of advanced drug delivery systems, has led to systems with exceptional advantages in terms of the therapeutic effect achieved. Many problems for administration of drugs, that are related with either the low stability and/or unfavorable physicochemical properties (high molecular weight, low permeability through biological barriers, low solubility), can be solved by innovative liposomal nanomedicines.
In the last years the use of liposomal systems for delivery of bioactive agents, such as growth factors, antibiotics, small molecule drugs, etc. as a strategy to enhance tissue regeneration, has been initiated. Liposomes may be used as independent drug delivery systems for administration of bioactives, or they may be associated/immobilized or embedded with or on different types of biomaterials (such as stents, catheters, injectable in situ forming gels, electrospun membranes, etc). The different possibilities, related methodologies, together with examples of the advantages towards tissue regeneration, will be analyzed.

Acknowledgement: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 874896

Keywords

Liposome; Regeneration; Targeting

Abstract **#2074**

CELLULAR SENESCENCE, SENOLYTICS, AND PROGENITOR FUNCTION: IMPLICATIONS FOR TISSUE REGENERATION AND TRANSPLANTATION

James L. Kirkland$_P$[1]

[1] *Mayo Clinic*

Corresponding author's email: kirkland.james@mayo.edu

Senescent cells (SC) accumulate in multiple tissues with aging and at etiologic sites of numerous diseases. Some SC release pro-apoptotic, inflammatory, tissue-damaging factors and spread senescence to normal cells, the “senescence-associated secretory phenotype” (SASP). SCs are resistant to apoptosis. SC can contribute to wound healing, but pre-existing or persistent SCs may delay this. SC and the SASP can cause stem cell/progenitor dysfunction. Adipose mesenchymal stem cell replication and differentiation into insulin-responsive adipocytes are interfered with by SASP factors. SCs interfere with cardiac and bone progenitor function and neurogenesis. Transplanting SCs into younger mice such that 1/10,000 cells are transplanted SCs causes frailty, accelerated onset of age-related diseases, and early mortality. Hearts transplanted from old into young mice function less effectively than hearts transplanted from young mice, related to mitochondrial DNA release by SCs in the old transplanted organ, inducing tissue dysfunction, immune activation, and rejection, further interfering with repair.

Senolytic agents promote apoptosis of SCs selectively. Intermittent senolytics alleviate multiple age- and chronic disease-related conditions in mice. Senolytics prevent frailty, accelerated chronic disease onset, and early death caused by transplanting SCs into younger mice. Senolytics improve function of hearts transplanted from old mice. In old mice, senolytics improve physical function, delay age-related diseases as a group, and increase remaining median lifespan. Early clinical trials demonstrated senolytics remove SCs from adipose tissue of diabetic humans and might reduce frailty in patients with idiopathic pulmonary fibrosis. Clinical trials of senolytics for several chronic diseases, geriatric syndromes, and impaired physical resilience are underway.

Abstract **#2075**

REPAIR VERSUS REGENERATION - CELL DELIVERY VIA A 3D PRINTED BIOMIMETIC WOUND DRESSING

Abbas Shafiee$_P$[1,2,3]

[1] *Herston Biofabrication Institute, Metro North Hospital and Health Service, Brisbane, QLD, 4029, Australia*

[2] *Royal Brisbane and Women's Hospital, Metro North Hospital and Health Service, Brisbane, QLD, 4029, Australia*

[3] *UQ Diamantina Institute, Translational Research Institute, The University of Queensland, Brisbane, Queensland, Australia*

Corresponding author's email: a.shafiee@uq.edu.au

Despite considerable research in developing biological wound dressings in enhancing the injury closure in the short term, their potential in reducing the formation of scars is limited. Here, using 3D printing we developed biomimetically designed medical-grade polycaprolactone (mPCL) dressings with critically adjusted pore size and anisotropic mechanical characteristic which favor skin wound healing. Melt electrowritten mPCL dressings were seeded with human gingival tissue multipotent mesenchymal stem/stromal cells (MSC) and cryopreserved using a clinically approved method of cryopreservation. Next, the regenerative potential of fresh or frozen cell-seeded mPCL dressing was assessed in a splinted full-thickness excisional wound in a rat model over six weeks. Application of mPCL dressings delayed the wound contraction and led to skin regeneration through granulation and re-epithelialization. Together, the use of bioactive mPCL dressings able to induce contractile forces and promote active wound closure with reduced scar tissue formation and ultimately improves patient outcomes.

Abstract **#2076**

RAMAN MICROSPECTROSCOPY AND RAMAN IMAGING IN REGENERATIVE AND PERSONALIZED MEDICINE

Katja Schenke-Layland[P][1,2,3]

[1] *NMI Natural and Medical Sciences Institute Reutlingen*

[2] *Dept. of Women's Health, University Hospital Tübingen*

[3] *Dep. of Medicine/Cardiology, University of California Los Angeles*

Corresponding author's email: katja.schenke-layland@uni-tuebingen.de

As the field of regenerative and personalized medicine matures and cell- or materials-based therapies as well as tissue-engineered products play an increasing role for health care strategies, the need for novel enabling technologies for the artefact-free, real-time characterization of cells, (bio)materials and tissue-engineered constructs in a more insightful, quantitative and preferably non-invasive manner becomes imperative. Raman microspectroscopy and Raman imaging have been established over the past 10 years to be suitable tools for the monitoring of the cell and tissue states in biological samples, assessing their biochemical and biomolecular structure marker-independently. With the addition of machine learning and artificial intelligence technologies Raman spectroscopy-based technologies will have the power to significantly improve diagnostic accuracy and speed, as well as provide reliable data for the improvement of cellular products and ATMP design optimization.

Keywords

extracellular matrix ; Raman microspectroscopy; FLIM

Abstract **#2077**

CLINICAL APPLICATION OF FIBRIN-AGAROSE BASED HUMAN BIOARTIFICIAL TISSUES

Antonio Campos[P][1,2]

[1] *Department of Histology and Tissue Engineering*

[2] *University of Granada. Spain*

Corresponding author's email: acampos@ugr.es

Several models of human bioartificial tissues and organs have been described and evaluated ex vivo. However, very few of these models have been translated to the clinical setting as advanced therapies medicinal products (ATMP). One of the biomaterials showing promising results is the combination of human fibrin and 0.1% agarose, which demonstrated to be highly biocompatible and fulfils the requirements for clinical translation. In this regard, the Tissue Engineering Group of the University of Granada was able to design and fabricate in the laboratory different types of bioartificial human tissues based on fibrin-agarose biomaterials, and performed a thorough preclinical characterization of these tissues for clinical use. As a result of this preclinical characterization, we were able to implement a clinical trial to evaluate a novel model of human bioengineered cornea in patients with severe corneal ulcers (ClinicalTrials.gov Identifier: NCT01765244), and we obtained approval from the Spanish National Medicines Agency for compassionate use of a model of human skin that is currently used for the clinical treatment of severely burnt patients. Preliminary results of both models are promising and support the clinical use of tissues generated by tissue engineering as ATMP.

Keywords

cornea; skin

Abstract **#2078**

STRATEGIES FOR 3D PRINTING COMPLEX CARDIAC TISSUE MIMICS

Brenda Ogle[P][1,2,3]

[1] *Department of Biomedical Engineering, University of Minnesota-Twin Cities*

[2] *Stem Cell Institute, University of Minnesota-Twin Cities*

[3] *Institute for Engineering in Medicine, University of Minnesota-Twin Cities*

Corresponding author's email: ogle@umn.edu

Cardiac tissue engineering has emerged as a means to create living, human, cardiac tissue outside the body as a model system in the near term and as a clinical replacement for diseased or damaged cardiac muscle in the long term. One goal of cardiac tissue engineering is the generation of a living, human pump in vitro that could replace animal models and eventually serve as an in vivo therapeutic. Models that replicate the geometrically complex structure of the heart, harboring chambers and large vessels with soft biomaterials, can be achieved using 3-dimensional bioprinting. Yet, inclusion of contiguous, living muscle to support pump function has not been achieved. We optimized a photo-crosslinkable formulation of native ECM (extracellular matrix) proteins and used this bioink to 3-dimensionally print human induced pluripotent stem cell-laden structures with 2 chambers and a vessel inlet and outlet. After human induced pluripotent stem cells proliferated to a sufficient density, we differentiated the cells within the structure and demonstrated function of the resultant human chambered muscle pump. Human chambered muscle pumps demonstrated macroscale beating and continuous action potential propagation with responsiveness to drugs and pacing. The connected chambers allowed for perfusion and enabled replication of pressure/volume relationships fundamental to the study of heart function and remodeling with health, disease, and interventional therapeutics.

Abstract **#2079**

mRNA-BASED THERAPEUTICS: NEW CLASS OF MEDICINES IN REGENERATIVE MEDICINE

Elizabeth Rosado Balmayor[P1,2]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands*

[2] *Musculoskeletal Gene Therapy Research Laboratory, Mayo Clinic, Rochester, USA*

Corresponding author's email: e.rosadobalmayor@maastrichtuniversity.nl

Increasing attention is being paid to the use of RNA as agents to facilitate tissue healing. Messenger RNAs (mRNAs) introduced into the cell induce synthesis of their encoded proteins. This approach holds advantages over traditional DNA-based gene therapy including improved cell internalization and higher transfection efficiencies, without disruption of the host genome. In the context of tissue engineering and regenerative medicine, the eventual turnover of the exogenous RNA is a further advantage. Because conventional RNAs have strong immunogenicity and low stability, chemical modifications are needed to facilitate their therapeutic application. Moreover, efficient delivery systems are of crucial importance for efficient cell internalization. This talk will cover progress on mRNA therapeutics with a focus on tissue engineering and regenerative medicine. Particular interest will be placed on applications of mRNA for musculoskeletal regeneration. The presentation will transit from mRNA biology and biochemistry to internalization of RNA using different biomaterials and non-viral delivery systems, ending with novel applications reported for the regeneration of diverse tissues. In terms of chemical modifications, use of modified nucleotides, UTRs, optimized poly(A) tails among others will be reviewed. Regarding RNA delivery, examples of novel lipid and polymer delivery systems will be shown. Also, advantages of mRNA internalization techniques (magnetofection) will be highlighted. Finally, examples of preclinical studies on mRNA applications to enhance the regeneration of tissues including bone and tendon will be discussed. Overall, this talk will give an overview of the cmRNA technology from basic aspects to preclinical proof of concepts.

Funding: H2020 No. 874790 (cmRNAbone).

Abstract **#2080**

DESIGN2HEAL: A JOURNEY FROM MACRO TO MICROSTRUCTURAL DESIGN CRITERA FOR EFFICIENT TOPOGRAPHIC IMMUNOMODULATION

Jürgen Groll[1], Tina Tylek[1,2], Matthias Ryma[1], Carina Blum[1]

[1] *University of Würzburg*

[2] *Drexel University*

Corresponding author's email: juergen.groll@fmz.uni-wuerzburg.de

Supplement-free induction of macrophage polarization solely through the topography of materials is an auspicious strategy but has so far significantly lacked behind the efficiency and intensity of media-supplementation based protocols. The project Design2Heal was initiated in 2013 with the aim to identify structural design criteria for the fabrication of scaffolds with strong topographic immunomodulation for human monocyte-derived macrophages. We started by applying Melt-Electrowriting (MEW) for the fabrication of fibrous 3D scaffolds made from poly(ε-caprolactone) (PCL) and advanced the precisely defined inter-fiber spacing from 100 µm down to 40 µm for a variety of pore geometries (rectangular, triangular and round). These scaffolds did facilitate primary human macrophage differentiation towards the M2 type, which was most pronounced for box-shaped pores with 40 µm inter-fiber spacing, but not with the desired efficiency. We then found that human monocyte-derived macrophages show a strong M2a like pro-healing polarization when cultured on type I rat-tail collagen fibers but not on collagen I films. Therefore, we hypothesized that highly aligned nanofibrils also of synthetic polymers, if packed into larger bundles in 3D topographical biomimetic similarity to native collagen I fibers, would induce a localized macrophage polarization. Through integration of flow directed polymer phase separation into MEW we developed Melt-Electrofibrillation, a process that yields nano-fiber bundles with a remarkable structural similarity to native collagen I fibers, particularly for medical grade PCL. These biomimetic fibrillar structures indeed induce a pronounced elongation of human monocyte-derived macrophages and unprecedentedly triggered their M2-like polarization similar in efficacy as IL-4 cytokine treatment.

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Abstract **#2081**

UNDERSTANDING CELL-MATRIX INTERACTIONS IN 3D CULTURE; DEFINE, REFINE AND COLLABORATE

Catherine Merry[P][1], Amanda Wright[2]

[1] *Stem Cell Glycobiology Group, Nottingham Biodiscovery Institute, University of Nottingham, UK*

[2] *Optics and Photonics Research Group, Faculty of Engineering; University of Nottingham, UK*

Corresponding author's email: cathy.merry@nottingham.ac.uk

Recently, we have seen a dramatic increase in groups using 3D culture to study cell behaviour. We now have a plethora of options for cell encapsulation and recent developments have provided elegant models incorporating one or more features such as multiple cell types, optogenetic control, microfluidics, high-throughput culture and complex analyses. For our group, the next challenge has been to understand what local interactions and forces encapsulated cells are experiencing. To achieve this, we have developed an interdisciplinary tool-box, allowing us to build then interrogate 3D cell-matrix models. We make use of induced pluripotent stem cell (iPSC) lines engineered to produce modified matrix, focusing primarily on glycosaminoglycans. We typically encapsulate these in a synthetic, self-assembling peptide hydrogel that we can functionalise with specific matrix components but which also allows us to study matrix deposition on a blank canvas background. We are also part of a team building a new instrument, that combines light sheet microscopy, optical trapping and multi-plane imaging (http://nu-sense.ac.uk). We can image live encapsulated cells in 3D at depth with good resolution over multiple days. If we incorporate beads into the gels, multiplane imaging allows us to record the Brownian motion of a bead in the z-axis, as well as in the x, y imaging plane, sensing the full 3D micro-rheology of the matrix. This allows us to probe local variations in the rheological properties of matrix close to the cells and to test the impact of differential matrix secretion by encapsulated cells, linking to their biological response.

Abstract **#2083**

CHALLENGES AND OPPORTUNITIES IN BIOENGINEERING AND BETA CELLS REPLACEMENT DEVICES

Aart van Apeldoorn[P][1,2]

[1] *Maastricht University*

[2] *MERLN institute for technology-inspired regenerative medicine*

Corresponding author's email: a.vanapeldoorn@maastrichtuniversity.nl

In the past decade a lot of new advances have been made regarding beta cell replacement devices. Developments in stem cell derived beta cell research have created opportunities to use these as an alternative to primary human islets. The consensus in the field is that beta cells still need to be delivered to patients with the help of a tailor-made implant. There is a current trend in the field where a shift in interest can be seen from immunoprotective closed devices, to open devices which allow the transplanted cell to be connected directly to the patient's vasculature. Both these strategies have advantages and disadvantages, the closed devices allow for protection against immune rejection omitting the use of immunosuppressive therapy, but with potential diminished mass transport to and from the transplanted cells, while the open devices allow for good mass transport and a more direct response of beta cells to changing glucose levels, but also come with the downside of immunosuppressive therapy. In the same time there is also growing interest in how perhaps the cell biomaterial interactions of materials used in these devices can be improved by the addition of biological factors. The goal is to give a comprehensive overview of the current trends and discuss opportunities how these challenges can potentially be addressed to improve beta cell replacement therapy to tackle type 1 diabetes.

Abstract **#2084**

BIOARTIFICIAL PANCREAS DEVICES FOR TREATMENT OF DIABETES – AN INTERDISCIPLINARY CHALLENGE

Barbara Ludwig[P][1]

[1] *University Hospital Carl Gustav Carus Dresden at the Technische Universität Dresden*

Corresponding author's email: barbara.ludwig@uniklinikum-dresden.de

The treatment options for diabetes mellitus have expanded considerably in recent years. In the next decade, a combined and concerted application of pharmacological, biotechnological and cell-based treatment strategies could revolutionize therapy. The decisive factor here is the interaction of fundamentally oriented and clinically active scientists in the fields of diabetology, cell biology, immunology and transplantation medicine, but also of materials scientists and biotechnologists. The common goal should be to establish individualized and safe forms of therapy for a broad patient population. The therapy goals are based on quality of life and prolongation of life without relevant late complications.
Islet transplantation has evolved into an established therapy for patients with type 1 diabetes. However, a widespread application is hampered by mandatory requirement of life-long immunosuppression, the shortage of cadaveric donor pancreata, and the limited lomg-term survival of transplanted cells. Therefore, eliminating immunosuppression, safe utilization of alternative insulin-producing cells and promoting long-term islet survival are key features for moving this therapy to larger scale application. Macroencapsulation devices as a delivery strategy could potentially target all three aims. Numerous encapsulation devices have been designed that combine immunoprotective properties and allow for free exchange of effector molecules. Major advances have been achieved in encapsulation technology, and some strategies are in translation to clinical application. However, there are still issues that need to be resolved associated with biocompatibility, graft oxygenation, immunprotection, inflammatory response, and the applicable islet mass.

Keywords

Bioartificial pancreas; islet transplantation; diabetes

Abstract **#2085**

ENGINEERED BIOMATERIALS FOR IN SITU TISSUE REGENERATION

Akhilesh K Gaharwar[P][1]

[1] *Texas A&M University*

Corresponding author's email: gaharwar@tamu.edu

Biomaterials engineered with tunable biophysical properties, specific biochemical cues, and complex architecture have emerged as powerful tools for regeneration medicine. These cues are required to induce regeneration by modulating extracellular microenvironment for in situ tissue repair. In this talk, I will outline some of the biomaterial approaches that we have developed to control and direct the body's regenerative capacity for tissue-specific regeneration. Specifically, we have utilized genome wide sequencing assays (transcriptomics) to understand complex cell-biomaterials interactions. These omics-based approaches provide an unbiased global view of the cellular activity with pivotal insights about the affected cellular pathways to optimize synthetic biomaterials for tissue engineering. In addition, I will discuss biomaterials loaded with bioactive cues that prime endogenous cells to perform tissue-specific regeneration. Furthermore, I will also show some of our recent work on 3D bioprinting to develop physiologically relevant tissue structures for disease modeling (vascular pathophysiology) and tissue engineering (bone and cartilage regeneration).

Abstract **#2086**

PERFUSION AND ENDOTHELIALIZATION OF ENGINEERED TISSUES WITH PATTERNED VASCULAR NETWORKS

Jordan S Miller[P][1]

[1] *Rice University*

Corresponding author's email: jmil@rice.edu

As engineered tissues progress toward therapeutically relevant length scales and cell densities, it is critical to deliver oxygen and nutrients throughout the tissue volume via perfusion through vascular networks. Furthermore, seeding of endothelial cells within these networks can recapitulate the barrier function and vascular physiology of native blood vessels. Here, we describe how to fabricate and assemble customizable open-source tissue perfusion chambers and catheterize tissue constructs inside them. Human endothelial cells are seeded along the lumenal surfaces of the tissue constructs, which are subsequently connected to fluid pumping equipment. The protocol is agnostic with respect to biofabrication methodology as well as cell and material composition, and thus can enable a wide variety of experimental designs. It takes ~14 h over the course of 3 d to prepare perfusion chambers and begin a perfusion experiment. We envision that this work will facilitate the adoption and standardization of perfusion tissue culture methods across the fields of biomaterials and tissue engineering.

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Abstract **#2087**

BIOENGINEERED IN VITRO 3D MODEL OF MYOTONIC DYSTROPHY TYPE 1 HUMAN SKELETAL MUSCLE

Xiomara Fernández-Garibay[P][1], María A. Ortega[1], Estefanía Cerro-Herreros[2,3,4], Jordi Comelles[1,5], Elena Martínez[1,5,6], Rubén Artero[2,3,4], Juan M. Fernández-Costa[1], Javier Ramón-Azcón[1,7]

[1] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), c/Baldiri Reixac 10-12, E08028 Barcelona, Spain

[2] University Institute for Biotechnology and Biomedicine (BIOTECMED), University of Valencia, Dr Moliner 50, E46100 Burjassot, Valencia, Spain

[3] Translational Genomics Group, Incliva Health Research Institute, Dr Moliner 50, E46100 Burjassot, Valencia, Spain

[4] Joint Unit Incliva- CIPF, Dr Moliner 50, E46100 Burjassot, Valencia, Spain

[5] Department of Electronics and Biomedical Engineering, University of Barcelona (UB), c/Martí i Franquès 1-11, E08028 Barcelona, Spain

[6] Centro de Investigación Biomédica en Red (CIBER), Av. Monforte de Lemos 3-5, Pabellón 11, Planta 0, E28029 Madrid, Spain

[7] Institució Catalana de Reserca I Estudis Avançats (ICREA), Passeig de Lluís Companys, 23, E08010 Barcelona, Spain

Corresponding author's email: xfernandezg@ibecbarcelona.eu

Myotonic dystrophy type 1 (DM1) is the most common hereditary myopathy in adults. The disease is characterized by progressive skeletal muscle degeneration that produces severe disability. There is still no effective treatment for DM1 patients, but new therapeutic strategies are being tested. Animal models and in vitro 2D cell cultures have been essential for these advances. However, these models cannot reproduce the biological complexity of the disease. Biofabrication tools can be applied to engineer human 3D culture systems that complement current preclinical research models.

Here, we describe the development of the first in vitro 3D model of DM1 human skeletal muscle. Patient-derived cells were encapsulated in micromolded gelatin methacryloyl-carboxymethyl cellulose methacrylate (GelMA-CMCMA) hydrogels through photomold patterning. These hydrogels present a microstructured topography that promotes myoblast alignment and differentiation, resulting in highly aligned myotubes from healthy and DM1 cells. The DM1 3D microtissues present the molecular alterations detected in patient biopsies. Importantly, fusion index analyses demonstrate that 3D micropatterning significantly improved DM1 cell differentiation into multinucleated myotubes compared to standard cell cultures. Moreover, characterization of the 3D cultures of DM1 myotubes detects a reduced thickness of myotubes that can be used for drug screening. Therefore, we evaluated the therapeutic effect of antagomiR-23b administration on bioengineered DM1 skeletal muscle microtissues. AntagomiR-23b treatment rescues both molecular DM1 hallmarks and structural phenotype, restoring myotube diameter to healthy control sizes. Overall, these new microtissues represent an improvement over conventional models and can be used as biomimetic platforms to establish preclinical studies for myotonic dystrophy.

Abstract **#2088**

DIGESTION DEGREE IS A KEY FACTOR TO REGULATE THE PRINTABILITY OF PURE TENDON DECELLULARIZED EXTRACELLULAR MATRIX BIO-INK IN EXTRUSION-BASED 3D CELL PRINTING

Fengyuan Zhao[P][1]

[1] *Department of sports medicine, Peking university third hospital.*

Corresponding author's email: fengyuanzhaopku@gmail.com

Improving the printability of pure, decellularized extracellular matrix (dECM) bio-ink without altering its physiological components has been a challenge in three-dimensional (3D) cell printing. To improve the printability of the bio-ink, we first investigated the digestion process of powdered dECM material obtained from porcine tendons. We manifested the digestion process of tendon derived dECM powders, which includes dissolution, gelatinization and solubilization. After a short dissolution period (around 10 min), we observed a 'High viscosity slurry' status (3 h) of the dECM precursors, i.e. the gelatinization process, followed by the solubilization processes, i.e. a 'Medium viscosity slurry' period (12 h) and a 'Low viscosity slurry' period (72 h). Although the 'Low viscosity slurry' status of the dECM bio-ink has been reported to be extrudable, it has poor printability. This study explores the printability of the 'High viscosity slurry' status of the dECM bio-ink, which has not been addressed thus far. The results demonstrate that this less digested status of the dECM bio-ink yields higher shape fidelity and stacking accuracy than the traditional over-digested status of the dECM bio-ink; this indicates better printability of this less digested dECM bio-ink. Bone marrow mesenchymal stem cells derived from rats was printed using the 'High viscosity slurry' status of the dECM bio-ink, yielding high cellular viability lasting for 7 d after printing. Thus, the 'High viscosity slurry' status of tendon dECM bio-ink can be utilized to fabricate complicated 3D organoid structures; it also shows promise for applications such as regenerative medicine and biomimetic tissue engineering.

Abstract **#2089**

FIRST STEPS TOWARDS A REPRESENTATIVE IN VITRO TENDON MODEL: EQUINE TENOCYTE SEEDING ON GELATIN HYDROGELS

Marguerite Meeremans[P][1], Lana Van Damme[2], Ward De Spiegelaere[3], Mario Van Poucke[4], Luc Peelman[4], Sandra Van Vlierberghe[2], Catharina De Schauwer[1]

[1] *Comparative Physiology, Faculty of Veterinary Medicine, Ghent University, Belgium*

[2] *Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Faculty of Sciences, Ghent University, Belgium*

[3] *Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Belgium*

[4] *Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Belgium*

Corresponding author's email: marguerite.meeremans@ugent.be

Tendinopathy is a common injury in human and equine athletes and the poor success with conventional therapy supports the need for novel treatments. Representative in vitro models are mandatory to facilitate translation of fundamental research into clinical therapies. Natural biomaterials provide favourable cell binding characteristics and are therefore appropriate to establish such a model. In this study, we seeded equine tenocytes onto four different hydrogels: methacrylated gelatin (gel-MA, 100% degree of substitution, DS), norbornene-functionalized gelatin (gel-NB, 85% and 55% DS) crosslinked with DTT and gel-NB (85% DS) crosslinked with thiolated gelatin (gel-SH, 75% DS). The physico-chemical properties (1H-NMR spectroscopy, gel fraction, swelling ratio, and storage modulus), cell characteristics (proliferation, viability, and morphology) and functionality (RT-qPCR) were evaluated and compared to tissue culture plastic (TCP). The thiol-ene based hydrogels showed a lower gel fraction/storage modulus and a higher swelling ratio compared to gel-MA. Viability on all hydrogels exceeded 95%, indicating excellent biocompatibility. At 14 days, significantly less tenocytes were observed on gel-MA compared to the thiol-ene crosslinked gelatin, and tenocytes on gel-NB85/DTT and gel-NB55/DTT showed the characteristic elongated morphology more in contrast to TCP and gel-MA. No significant difference in extracellular matrix gene expression (COL1A1, COL3A1, TNC & DCN) was observed on gelatin discs vs TCP, confirming the suitability of gelatin for tenocyte culture. In conclusion, thiol-ene crosslinked gelatins are preferred over gel-MA when considering physico-chemical characteristics and cell responses. These data improve our knowledge on the interaction between natural biomaterials and tenocytes, essential to establish a representative tendon model.

Keywords

In vitro tendon model; Hydrogels

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Abstract **#2090**

AN ECHO IN CARTILAGE BIOLOGY: COMBINATORIAL TREATMENTS INDUCE A SWITCH BETWEEN TRANSIENT AND PERMANENT CARTILAGE PHENOTYPES

Sakshi KhuranaP[1], Jacqueline Plass[1], Stefano Schivo[2], Marcel Karperien[1], Janine Post[1]

[1] *Department of Developmental BioEngineering, Technical Medicine Centre, University of Twente, Enschede, The Netherlands.*

[2] *Department of Computer Science, Open University, Heerlen, The Netherlands*

Corresponding author's email: j.n.post@utwente.nl

One of the fundamental questions in cartilage biology is: what determines the switch between permanent cartilage found in the articular joints and transient hypertrophic cartilage in the growth plate that functions as a template for bone? This switch is observed both in a subset of osteoarthritis patients that develop osteophytes, as well as in cell-based tissue engineering strategies for joint repair. Cell based cartilage repair strategies suffer from inefficient chondrogenesis and hypertrophy1,2. A thorough understanding of the mechanisms regulating cell fate will provide opportunities for treatment of cartilage disease as well as in tissue engineering strategies.

Because of the inherent complexity of regulatory networks, they cannot be efficiently analysed and understood without computational assistance. To obtain insight into the function of such complex networks we developed a dynamic computational model of chondrocytes, the Executable CHOndrocyte or ECHO3. This signaling network integrates 8 signal transduction pathways: WNT, BMP, TGFβ, IHH, IGF, PTHrP, HIF1, and the FGF. The entire network is represented by ~100 nodes and ~300 interactions. This model gives output in the form of SOX9+ and RUNX2+ active states, representing the permanent articular and hypertrophic phenotype of chondrocytes, respectively.

We used model checking in ECHO to predict combinatorial treatments that induce the switch from a transient cartilage to permanent cartilage and vice versa. Here, we show experimental validation of the computer model predictions ex vivo (metatarsals) and in vitro (cell culture) and provide insight into processes determining cartilage cell fate.

Keywords

signal transduction; articular cartilage; SOX9

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Abstract **#2091**

DEVELOPMENT OF SPECIAL PROCESSING DEVICES FOR BIOFABRICATION OF VASCULAR TISSUES

Yasutaka Takehana[P][1], Norihiro Minamii[1], Shintaroh Iwanaga[2], Makoto Nakamura[2]
[1] *Graduate School of Science and Engineering for Education, University of Toyama*
[2] *Graduate School of Science and Engineering for Research, University of Toyama*
Corresponding author's email: yasutakatakehana@gmail.com

In tissue engineering, research and development of biofabrication is attracting attention as one of the promising approaches for producing tissues and organs with the aids of computers and machines. However, several multiple processing steps are required to produce hierarchal, matured and functional tissues and organs. In this study, we designed and prototyped the devices that would carry out several essential processes for producing hierarchal vascular grafts as follows; scaffold fabrication, cell seeding, assembly, culture, and maturation. At first, we designed and prepared the support for fabricating tubular structures using 3D printer, and then, prototyped the coating devise for covering the support with photo-crosslinkable hydrogel, gelatin methacrylate (GelMA). Using this device, pre-gel of GelMA was uniformly coated by rotating the support. After coating, a stiff tube-shaped gel scaffold was obtained by UV irradiation. Next, we prototyped the cell seeding device, which enables cell seeding rotationally inside a tubular gel scaffold and cell culture subsequently. After seeding endothelial cells, cell adhesion throughout the inner wall of the tubular scaffold was recognized on day 3. In our previous study, we made cell fibers as bioparts. And we prototyped an assembly device to wind up the cell fiber. Using this device, we attempted to reproduce the multi-layered structure of vascular tissue by wrapping cell fibers around a GelMA pre-coated support and then culturing it. Finally, endothelial cells were seeded into the structural lumen. To construct a vascular tissue with a multi-layered structure, bioprocessing technology is required to carry out a number of necessary steps.

Abstract **#2092**

PEG-FIBRIN GEL AS A SCAFFOLD FOR VOCAL FOLD SCAR CELL THERAPY: IN VIVO EXPERIMENTAL STUDY

Mikhail V. Svistushkin[P][1], Svetlana V. Starostina[1], Anastasia I. Shpichka[2], Peter S. Timashev[2], Anatoliy B. Shekhter[2], Valeriy M. Svistushkin[1], Alexey Fayzullin[2]

[1] *Department for ENT Diseases, Sechenov First Moscow State Medical University (Sechenov University)*

[2] *Institute for Regenerative Medicine, Sechenov First Moscow State Medical University (Sechenov University)*

Corresponding author's email: swistushkin@yandex.ru

The study aim was a preclinical in vivo estimation of cell product based on human bone marrow MSCs and polyethylene glycol (PEG)-fibrin gel in vocal fold scar restoration.

We unilaterally injected 0,5ml of 5:1 ratio PEG-fibrin gel containing 5 x 105 MSCs (MSCs+GEL) into the rabbit vocal fold wound after excision of the mature scar. In control groups: MSCs in solution (MSCs), gel without cells (GEL), saline solution (SS) were similarly implanted (n=6 for each group). During the operation and post-operatively, bleeding intensity and respiratory disorders were assessed (point-scale); three days post implantation - cell survival rate, in three months, after animals painless sacrificing - vocal folds morphology and tissue Young's modulus.

In the MSCs+GEL and GEL groups the bleeding intensity was significantly lower comparing with MSCs and SS ($p=0.03945$), while the risk of the respiratory disorders in all groups was out of significant difference. On the third day cell survival rate was higher in the MSCs+GEL group. Morphology criteria analysis revealed significant difference between MSCs+GEL and SS groups in collagen fibers irregularity, density, muscle tissue fibrosis, with alteration predominance in SS ($p<0.05$). Lamina propria thickness in SS group was significantly higher when compared to MSCs, GEL ($p<0.05$) and MSCs+GEL ($p<0,0001$). Young's modulus in MSCs and MSCs+GEL was significantly lower comparing to group SS ($p<0.05$).

PEG-fibrin gel scaffold for MSCs reduces the intraoperative bleeding without respiratory disorders increase, facilitates cell surviving and improves the restoration of vocal fold morphology and mechanics. This study was financially supported by RSF № 21-15-00339.

Abstract **#2093**

CULTURING PATIENT-DERIVED MALIGNANT HEMATOPOIETIC STEM CELLS IN ENGINEERED AND FULLY HUMANIZED 3D NICHES

Andres Garcia-Garcia[P][1], Thibaut Klein[1], Gordian Born[1,2], Morgane Hilpert[1], Arnaud Scherberich[1,2], Claudia Lengerke[3], Radek C. Skoda[4], Paul E. Bourgine[5,6], Ivan Martin[1,2]

[1] Tissue Engineering, Department of Biomedicine, University Hospital Basel, University of Basel, 4031 Basel, Switzerland

[2] Technologies for Tissue Engineering, Department of Biomedical Engineering, University of Basel, 4123 Allschwil, Switzerland

[3] Stem Cells and Hematopoiesis, Department of Biomedicine, University Hospital Basel, University of Basel, 4031 Basel, Switzerland

[4] Experimental Hematology, Department of Biomedicine, University Hospital Basel, University of Basel, 4031 Basel, Switzerland

[5] Laboratory for Cell, Tissue, and Organ Engineering, Department of Clinical Sciences, Wallenberg Center for Molecular Medicine, Lund University, 22100 Lund, Sweden

[6] Stem Cell Center, Lund University, 221 00 Lund, Sweden

Corresponding author's email: andres.garcia-garcia@unibas.ch

Human malignant hematopoietic stem and progenitor cells (HSPCs) reside in bone marrow (BM) niches, which remain challenging to explore due to limited in vivo accessibility and constraints with humanized animal models. Several in vitro systems have been established to culture patient-derived HSPCs in specific microenvironments, but they do not fully recapitulate the complex features of native bone marrow. Our group previously reported that human osteoblastic BM niches (O-N), engineered by culturing mesenchymal stromal cells within 3D porous scaffolds under perfusion flow in a bioreactor system, are capable of maintaining, expanding and functionally regulating healthy human cord blood-derived HSPCs. Here, we first demonstrate that these 3D osteoblastic niches can sustain malignant CD34+ cells from acute myeloid leukemia (AML) and myeloproliferative neoplasms (MPN) patients for up to 3 weeks. Moreover, human malignant cells distribute in the bioreactor system mimicking the spatial distribution found in native BM tissue, where most HPCs remain linked to the niches and mature cells are released to the circulation. In order to exemplify the possibility to engineer environments with customized features, we used human adipose tissue-derived stromal vascular fraction (SVF) cells to generate a stromal-vascular niche (SV-N). Our findings indicate that the O-N and SV-N provide different niche signals that reduce leukemic UCSD-AML1 cell expansion while promoting differentiation. The possibility to culture patient-derived malignant hematopoietic cells within customizable microenvironments capturing the complexity and cellular diversity of human BM niches could be exploited to investigate mechanisms of blood cancer development and to test effectiveness of personalized therapeutic strategies.

Keywords

Malignant hematopoiesis; 3D niches; Perfusion bioreactors

Abstract **#2094**

CHARACTERIZATION OF EXTRACELLULAR VESICLES FROM PORCINE, CANINE AND HUMAN NOTOCHORDAL CELL-CONDITIONED MEDIUM

Josette C. van Maanen[P][1], Frances C. Bach[1], Markus Templin[2,3], Marca H.M. Wauben[4], Marianna A. Tryfonidou[1]

[1] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*

[2] *NMI TT Pharmaservices, Berlin, Germany*

[3] *NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany*

[4] *Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands*

Corresponding author's email: j.c.vanmaanen@uu.nl

Low back pain (LBP) episodes are common and affect everyday life. A major cause for chronic LBP is intervertebral disc degeneration. Notochordal cells (NCs) possess regenerative potential that could be exploited for therapeutic approaches. Extracellular vesicles (EVs) secreted by NCs may mediate this effect. To facilitate proper EV characterization a bead-based western technology was conducted to identify NC-derived EV-associated protein markers.

NC-conditioned medium (NCCM) was generated by culturing NC-rich tissue of porcine, canine, and human origin. EVs were isolated through differential centrifugation followed by size exclusion chromatography and characterized using DigiWest technology, a high-throughput bead-based multiplex platform. DigiWest analysis of porcine and canine NCCM-derived EVs revealed the presence of 12 proteins in common. In human NCCM-derived EVs, only 2 proteins were identified, most probably due to technical limitations related to low starting protein quantities. These proteins were also present in porcine/canine NCCM. Other proteins that can be recovered with EVs (co-isolated or contaminant proteins) were not detected, while they were detectable in positive control samples, indicating that they were absent in EV samples.

Altogether, for porcine and canine NCCM-derived EVs, we identified transmembrane, GPI-anchored, and cytosolic proteins that are required for EV characterization according to the standards of the International Society for Extracellular vesicles. Isolated EVs were devoid of contamination based on the absence of non-vesicular components. Based on these results, a panel of 19 proteins was composed for characterization of NC-derived EVs from different species.

This project received funding from the European Union's Horizon 2020 program iPSpine (No. 825925).

Keywords

Notochordal cells; Extracellular vesicles; Digiwest technology

Abstract **#2095**

ANALYSIS OF SAPHENOUS VEIN GRAFTS IN CABG LUMEN DIAMETER, WALL THICKNESS AND INTIMA MEDIA THICKNESS RATIO

ATIQAH HARON[P][1]

[1] *Center of Tissue Engineering and Regenerative Medicine*

Corresponding author's email: atiqahnur717@gmail.com

Background and Aims: Saphenous vein (SV), are the most used graft covering around 70-75% of coronary bypass grafting (CABG). Unfortunately, SV graft patency are proven to be poor with 50% graft failure observed at 5 years after CABG bypasses. We hypothesized that existing intimal hyperplasia SV contributes to its lower patency. Therefore, this study aims to analyses the prevalence of intimal hyperplasia in SV graft collected from CABG surgery. Methods: Surplus SV from CABG surgery at Hospital Canselor Tuanku Mukhriz (HCTM) were collected and the lumen diameter, intima media thickness (IMT) and intimal hyperplasia ratio were quantified via histological sections stained with H&E and EVG. Results: The veins were successfully fixing, processing, embedding and cutting. The thickness of the sample approximately 3-5 µ? were cut by using microtome. We found that all the vein samples show differences in the morphology either lumen area or wall thickness. Most of the sample's diameter were about 12.5mm to 18.5mm length. However, four samples were outliers either the vein have thicker intimal hyperplasia or none of it. IMT between samples were not as homogenous as diameter observed. The thickness of intimal hyperplasia prominent in some samples where the length was measured up to >0.4mm in length compared to others. Conclusions: Most of the samples collected was not too healthy as the patency of graft from CABG surgery will be decrease. The inconsistency between diameter of vessel and IMT variation shows the possible formation of intimal hyperplasia.

Abstract **#2096**

BIOACTIVE SCAFFOLDS FOR THE REGENERATION OF THE EPITHELIAL LINING THROUGH GUIDANCE FROM BASEMENT MEMBRANE PROTEIN

Dina Gadalla$_P$[1], Yourka Tchoukalova[1], David Lott[2]

[1] *Mayo Clinic Head and Neck Regenerative Medicine Laboratory*

[2] *Mayo Clinic Department of Head and Neck Surgery, Division of Laryngology*

Corresponding author's email: gadalla.dina@gmail.com

Almost all tissues consist of an epithelial lining which can be key to the appropriate function of organs. Among these organs are esophagus, trachea, cornea and skin. A lack of epithelium in hollow organs can lead to stenosis, collapse and infection that restrict patency. Hence, to successfully engineer artificial tissue for clinical application, it is crucial to regenerate a functional epithelium. This can be achieved by providing surfaces that are favorable to the migration and polarization of epithelial cells. Here, we show that the incorporation of a thin fiber layer with an adsorbed protein mixture can guide epithelial cell response. Specifically, aligned polycaprolactone/heparin blend fibers are electrospun then adsorbed with a mixture of collagen IV and laminin proteins to mimic both the physical properties and chemical composition of the native basement membrane. Our in vitro cell studies show that these constructs modulate better attachment of epithelial cells, their migration to the scaffold structure, and an increased rate of epithelial cell differentiation. Furthermore, electrospinning on a widely used implant (Medpor, stryker) for head and neck reconstruction resulted in a well integrated composite where the fibers were well adhered to the implant surface. Thus, this fiber layer construct is a component that can be incorporated to the epithelial surface side of tissue engineered replacements to help with the epithelialization of large surface areas.

Abstract **#2097**

ANALYSIS OF SAPHENOUS VEIN GRAFTS IN CABG LUMEN DIAMETER, WALL THICKNESS AND INTIMA MEDIA THICKNESS RATIO

ATIQAH HARON$_P$[1], Muhammad Dain Yazid[1], Mohd Ramzisham Abdul Rahman[2], Nadiah Sulaiman[1]

[1] *Center of Tissue Engineering and Regenerative Medicine*

[2] *Department of Surgery, Hospital Canselor Tuanku Mukhriz*

Corresponding author's email: atiqahnur717@gmail.com

Background and Aims: Saphenous vein (SV), are the most used graft covering around 70-75% of coronary bypass grafting (CABG). Unfortunately, SV graft patency are proven to be poor with 50% graft failure observed at 5 years after CABG bypasses. We hypothesized that existing intimal hyperplasia SV contributes to its lower patency. Therefore, this study aims to analyses the prevalence of intimal hyperplasia in SV graft collected from CABG surgery. Methods: Surplus SV from CABG surgery at Hospital Canselor Tuanku Mukhriz (HCTM) were collected and the lumen diameter, intima media thickness (IMT) and intimal hyperplasia ratio were quantified via histological sections stained with H&E and EVG. Results: The veins were successfully fixing, processing, embedding and cutting. The thickness of the sample approximately 3-5 µ? were cut by using microtome. We found that all the vein samples show differences in the morphology either lumen area or wall thickness. Most of the sample's diameter were about 12.5mm to 18.5mm length. However, four samples were outliers either the vein have thicker intimal hyperplasia or none of it. IMT between samples were not as homogenous as diameter observed. The thickness of intimal hyperplasia prominent in some samples where the length was measured up to >0.4mm in length compared to others. Conclusions: Most of the samples collected was not too healthy as the patency of graft from CABG surgery will be decrease. The inconsistency between diameter of vessel and IMT variation shows the possible formation of intimal hyperplasia.

Keywords

CABG; Saphenous vein; Intimal hyperplasia

Abstract **#2098**

EVALUATION OF DIFFERENTIATION POTENTIAL OF CHICKEN AND MOUSE EMBRYONIC MESENCHYMAL STEM CELLS IN OVO

Azadeh JahanbakhshP[1], Bart smeets[2], Liesbet Geris[1,3,4,5], Ioannis Papantoniou[1,6], Przemko Tylzanowski[1]

[1] *Skeletal Biology and Engineering Research Center, KU Leuven, Belgium*

[2] *Mechatronics, Biostatistics and Sensors (MeBioS), Leuven (Arenberg), Kuleuven University*

[3] *Institute of Chemical Engineering Sciences, Foundation for Research and Technology – FORTH, Patras, Greece*

[4] *Biomechanics Section, KU Leuven, Belgium*

[5] *Prometheus Division of Skeletal Tissue Engineering, Department of Materials Science, KU Leuven University*

[6] *Prometheus Division of Skeletal Tissue Engineering, Department of Materials Science, KU Leuven University*

Corresponding author's email: azadeh.jahanbakhsh@kuleuven.be

Recombinant limb engineering is a technique that allows the analysis of the morphogenetic potential of cells derived from limb bud mesenchyme under the ectodermal influence. Mammalian cells grafted in an avian environment respond fully to the local molecular cues and differentiate along the predicted pathways. We use the recombinant limb technique to produce an organized higher structure from recombinant limbs of chicken and mouse mesenchymal cell aggregates by incorporating limb bud mesenchymal cells from wild-type mice and chicken ectodermal jacket of the chicken.

The mesenchymal stem cells are isolated from the limb bud mesenchyme of chicken embryos at HH stages 19-23 and then inserted into the chicken ectodermal jacket collected from the same stage embryos. Next, the recombinant limb is transplanted into the flank region of the chicken embryo at HH stage 19-23 or placed on the chorioallantoic membrane.

The growing limbs will be characterized using histological staining and in situ hybridization methods to monitor the efficiency of the ectopic limb development.
In the next steps, mouse limb bud mesenchyme and eventually human-derived iPSC will be evaluated in this model system.

Abstract **#2099**

BIOFABRICATION OF BIFUNCTIONAL HYDROGEL SCAFFOLDS FOR NEURAL TISSUE DAMAGE REPAIR

Ozgul Gok$_P$[1], İleyna Sevde Uvak[1]
[1] *ACIBADEM MEHMET ALI AYDINLAR UNIV*
Corresponding author's email: ozgul.gok@acibadem.edu.tr

INTRODUCTION:
3D hydrogels with physically or chemically cross-linked networks and high water-uptake capacity can be utilized as artificial cell-hosting scaffolds together with being ideal porous structures for sustained therapeutics release. In this study, UV-curable natural polymer based hydrogels were prepared and loaded with an anti-inflammatory drug for neural tissue damage repair and also immobilized with a neural-cell adhesion peptide for specific cell adhesion.1
METHOD:

Methacrylated Hyaluronic acid (HA-MA) polymer was synthesized and cross-linked under UV light (365nm) to obtain hydrogels with compatible stiffness for neural tissue. For nerve repair, anti-inflammatory drug, Ibuprofen, was encapsulated into this structure for sustained release. During 3D-Bioprinting process of this hydrogel, methacrylated IKVAV peptide was introduced into hydrogel structure in a patterned structure.
RESULTS:
IKVAV-immobilized and hyaluronic acid-based hydrogels were prepared and successfully 3D-printed in aligned pattern structure for neural cell adhesion. Also ibuprofen was loaded with a high capacity for neural tissue damage repair.
DISCUSSION AND CONCLUSION:
The obtained hydrogels were characterized by FT-IR for their functional groups and mechanical behavior was analyzed by reometer. SEM results indicated that all hydrogels have porous structure and they were shown to liberate drug molecules slowly within a week. Peptide immobilized regions of this hydrogel scaffold provides selective adhesion for nerve cells compared to fibroblasts.
ACKNOWLEDGEMENTS:
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Abstract **#2100**

MECHANOBIOLOGY OF TISSUE REPAIR

Viola Vogel[P][1,2]

[1] *ETH Zurich*

[2] *Laboratory of Applied Mechanobiology*

Corresponding author's email: viola.vogel@hest.ethz.ch

From the first steps in blood coagulation all the way to functional tissue repair, how to recreate homeostasis after injury or disease is a holy grail in Regenerative Medicine. While the roles of niche-specific biomarkers, transcriptomic, epigenetic profiles, as well as secretomes in directing cell-cell communication have been broadly studied, cells furthermore utilize a range of physical stimuli to communicate with each other and their environments as revealed by the rapidly growing fields of Mechanobiology and Nano-/Mechanomedicine. Bidirectional mechanical communication through cell-cell junctions, cell-ECM linkages and ECM fiber stretching is a major coordinator to either maintain homeostasis, or drive cell niches towards tissue repair or pathological transformations. Towards guiding regenerative processes, we now discovered that platelets not just initiate thrombus formation after injury, but that they actively assemble the first provisional fibronectin matrix using the platelet integrin αIIbβ3, instead of α5β1 as fibroblasts do. As cells pull on ECM fibers, they stretch them which can activate or destroy crucial binding sites for other ECM components and cytokines. With a newly developed peptide probe, we now discovered that the fibronectin fibers are kept under high tension in most healthy organ tissues probed, but are structurally relaxed or proteolytically cleaved in inflamed tissues, including cancer and virally infected lymph nodes. To understand the underpinning mechanisms how the mechano-communication of cells with ECM fibers directs either the maintenance of tissue homeostasis, or if perturbed drives pathological ECM transformations, we will also report on our recent insights obtained from de novo grown microtissue platforms.

Abstract **#2101**

LASER CREATION OF BIOPOLYMERS STRUCTURES WITH NETWORKS OF SINGLE-WALLED CARBON NANOTUBES FOR CARDIOVASCULAR APPLICATIONS

Alexander Yu. Gerasimenko[1,2]

[1] *National Research University of Electronic Technology MIET, Head of the Biomedical Nanotechnology Laboratory*

[2] *I.M. Sechenov First Moscow State Medical University, Head of the Biomedical Nanotechnology Laboratory*

Corresponding author's email: gerasimenko@bms.zone

In recent times biophotonic techniques are often used in applications of tissue engineering. A promising approach is laser creation of new implant materials, which stimulate the proliferation of cells during tissue formation. At present, technologies are being actively developed to replace lost or poorly functioning human tissues with biocompatible and bioactive artificial structures. The need for such structures is especially acute in the case of cardiovascular diseases. This work proposes the research physicochemical and biological properties of biopolymer structures: albumin, collagen, and chitosan with networks of single-walled carbon nanotubes. The morphology features for the biopolymer structures, which are fabricated due to the phase transition of aqueous dispersions under the action of laser radiation, have been determined. The biopolymer structures fabricated by laser demonstrated conductivity that were higher (12.4 S/m) than those for structures by thermostat (4.7 S/m). The hardness of the biopolymer structures by laser was 482±10, 425±10, and 407±15 MPa for albumin, collagen and chitosan, respectively. The hardness of the thermostat layers was less than 100 MPa. The viability of endothelial cells и cardiac fibroblasts in biopolymer structures was improved. The biopolymer structures ensured a normal level of hemolysis during interaction with erythrocytes. Thus, biopolymer structures can be fabricated in various shapes to create independent implantable tissue-engineering structures in the heart or coatings for cardiovascular devices. This is proved by the results of studies of the morphology, electrical conductivity, as well as the bio- and hemocompatibility of the layers.

Abstract **#2102**

INFLUENCE OF SCAFFOLD GEOMETRY ON BMP-2 INCORPORATION AND IN VIVO BONE REGENERATION

Charlotte Garot$_P$[1], Julie Vial[2,3], Paul Machillot[1], Adeline Decambron[2,3], Delphine Logeart-Avramoglou[2,3], Julien Vollaire[4,5], Véronique Josserand[4,5], Emeline Maurice[2,3], Véronique Viateau[2,3], Mathieu Manassero[2,3], Georges Bettega[4,6], Catherine Picart[1]

[1] *CEA, CNRS, Université de Grenoble Alpes, ERL5000 BRM, IRIG Institute, 17 rue des Martyrs, F-38054, Grenoble, France*

[2] *Université de Paris, CNRS, INSERM, B3OA, F-75010 Paris, France*

[3] *Ecole Nationale Vétérinaire d'Alfort, B3OA, F-94700 Maisons-Alfort, France*

[4] *INSERM U1209, Institut Albert Bonniot, F-38000 Grenoble, France*

[5] *Université Grenoble Alpes, Institut Albert Bonniot, F-38000 Grenoble, France*

[6] *Service de chirurgie maxillo-faciale, Centre Hospitalier Annecy Genevois, 1 avenue de l'hôpital –74370 Epagny Metz-Tessy, France*

Corresponding author's email: charlotte.garot@cea.fr

Critical-size bone defects are unable to heal by themselves. Autologous bone graft is the gold standard to treat such defects, but it shows some limitation. To address this issue, we designed three scaffolds (cylinders 25mm long and Ø14mm) with different geometries to repair a long-segmental critical-size defect in a sheep metatarsal bone: (i) geometry Lines with 880µm cubic pores and a central cylinder hole (Ø5mm) ; (ii) geometry Gyroid, with 1mm gyroid pores and a central cylinder hole of Ø5mm ; (iii) geometry Double Lines, with 1.2mm cubic pores in the outer of the cylinder while its center (Ø7mm) had a gyroid pore shape with 2mm pores. The three geometries all presented a different effective surface. The 3D-printed scaffolds made of clinical-grade PLA were coated with a polyelectrolyte film and loaded with BMP-2 at a targeted surface dose of 9.2 µg/cm². BMP-2 quantification showed that the geometry Lines had the lowest incorporated BMP-2 surface dose while the geometry Gyroid had the highest. Surprisingly, the scaffolds with different surfaces had incorporated similar total quantity of BMP-2, suggesting that scaffold geometry influenced BMP-2 incorporation in the polyelectrolyte film. Interestingly, X-ray radiographs, µCT scans, and histology showed that despite its lowest incorporated BMP-2 surface dose, the geometry Lines presented the fastest and strongest bone formation. The geometry Double Lines did not allow bone repair. As the total quantity of BMP-2 was similar in every scaffolds, this suggests that the scaffold geometry influenced bone regeneration kinetics and the amount of new bone formed.

Keywords

Bone tissue engineering; 3D printing; bone morphogenetic protein 2

Abstract **#2103**

STRONTIUM DOPED B-TCP-PLGA SCAFFOLD FOR THE REPAIR OF CRITICAL-SIZED DEFECTS

Samuel Tiplady$_P$[1], Krishna Manda[1], Fraser Buchanan[1], Alex Lennon[1]

[1] *Queen's University Belfast*

Corresponding author's email: stiplady01@qub.ac.uk

The development of bioresorbable scaffolds to treat critical-sized defects has been widely studied; a generally accepted consensus on desirable properties has been achieved but the material selection has not. The gold standard for the treatment of bone defects, autologous bone grafts, has had relative success however there are fundamental weaknesses, namely lack of native bone and possible donor site morbidity. 3D printing using thermoplastics such as poly(e-caprolactone) (PCL), poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA) has allowed for the development of tuneable patient-specific bio-scaffolds that can vary in architecture, resorption time, and mechanical properties. However, these thermoplastics lack the osteoconductivity and osteoinductivity necessary to regenerate sufficient bone for full healing. Therefore, the development of a composite osteogenic scaffold is necessary. This research aims to produce a 3d printed PLGA scaffold containing strontium doped TCP filler which will enhance bone formation in bone defects. Strontium, an alkaline earth metal, has high biological relevance in tissue regeneration, it has been shown to increase mineralization and osteogenic markers in multiple studies. This combined with β-TCP, proven to be biocompatible and osteoconductive, has the potential to overcome issues arising from pure thermoplastic scaffolds. Three scaffolds of varying Sr-TCP wt% (0, 10, 20wt%) will be produced with analysis on the physio-chemical, mechanical, and biological performance. Including characterization of the compressive and Youngs' modulus, cell culture tests (on the effect on mineralization and osteogenic markers) as well as in vitro degradation studies.

Keywords

Strontium; 3d printing; scaffold

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Abstract **#2104**

BIOFABRICATION OF PHOTO-CROSSLINKED CONSTRUCTS WITH HIGHLY ALIGNED MICROSTRUCTURE FOR TISSUE ENGINEERING

Hao Liu[P][1], Riccardo Rizzo[1], Marcy Zenobi-Wong[1]

[1] *Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences & Technology, ETH Zürich*

Corresponding author's email: hao.liu@hest.ethz.ch

Biomaterials with cell instructive properties are key to being able to develop highly functional aligned tissues like muscle, tendon and nerves. Highly aligned fibers have been achieved by electrospinning and melt electrowriting techniques. However, fabrication of these fiber use solvents and/or high voltage which is not compatible with presence of cells, and cells cannot be encapsulated within the scaffold subsequently [1, 2]. Extrusion-based bioprinting has been performed to create highly aligned polymer strands [3], but these strands are generally several hundred microns in diameter which are too large to have significant cell guiding properties. Two-photon polymerization methods can produce 3D models with subcellular resolution, but the manufacture of large structures in the presence of living cells is challenging with poor cell biocompatibility [4]. Here we have developed a method to print 3D hydrogel structures which are composed of highly aligned 10-30 µm diameter microbeams using photo-crosslinked bioresin. The microbeams are similar in size to the cell, which provides physical cues for cell alignment. These printed structures are highly customizable on a macro level and precise control of multi-cell type and/or multi-materials can be achieved. Moreover, the aligned microbeams provide the printed structure with ideal mechanical properties that allow for the application of tensile loading required for the maturation of muscle and tendon tissue. Finally, the encapsulated human fibroblasts showed highly viable (>95%) and confirmed the production of highly aligned collagen fibers under the guidance of microbeams. This biofabrication approach shows great promise for multiple applications including fascicular tissue engineering.

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Abstract **#2106**

SYNTHESIS AND CHARACTERIZATION OF 3D GELMA CORE-SHELL MICROSPHERES

Pei Leng Tan[P][1], Lay Poh Tan[1]

[1] *School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore.*

Corresponding author's email: peileng001@e.ntu.edu.sg

Since the turn of the last century, the development of three-dimensional (3D) microspheres have piqued the attention of researchers due to the ability to better mimic the cellular microenvironment in-vivo for regenerative medicine purposes. The traditional 3D microspheres are generally made of a single material and lacks compartmentalisation that can truly replicate the several different cells present in an organ. Herein, we attempt to fabricate core-shell microspheres made of gelatin methacryloyl (GelMA) where the outer shell layer could serve to protect the encapsulated cells while allowing for the co-culture of different cell types for different biomedical applications. GelMA with varying degree of methylacrylate substitution was first synthesized using the protocol published by our group1. The successful conjugation of methacrylamide grafts to gelatin was confirmed by 1H-NMR and rheological studies of GelMA were done to characterise the storage moduli and viscoelastic property of GelMA with different degree of substitution (DS). A nearly monodisperse 3D GelMA core-shell microspheres with a size distribution of 395 ± 25µm were successfully fabricated via co-axial electrospraying using two-fluid GelMA solutions. This facile core-shell electrospray method thus renders this system useful to encapsulate and co-culture different cells in a scalable manner for cell therapeutics and regenerative medicine purposes.

Keywords

3D Microspheres; Core-Shell; Electrospray

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Abstract **#2107**

REJUVENATION OF MESENCHYMAL STEM CELLS BY LIPOSOME NANOCARRIER INDUCING MITOPHAGY

Keisuke Ashiba$_P$[1], Takeru Yamazaki[1], Koki Mino[1], Kiyoshi Sato[1], Hiroyoshi Kawakami[1]

[1] *Tokyo Metropolitan University, Department of Applied Chemistry*

Corresponding author's email: ashiba-keisuke@ed.tmu.ac.jp

Mesenchymal stem cells (MSCs) - based therapy has been widely used for therapeutic application of various diseases. MSCs have a self-renewal ability and multiple differentiation potential. When cultured MSCs are used in clinical, cellular senescence of MSCs is progressed because MSCs are repeatedly cultured in vitro. To get many MSCs without cellular senescence, the senescence should be suppressed in culturing process. To suppress the cellular senescence, many strategies had been tried, but rejuvenation of cells was not accomplished. In senescent cells, many dysfunctional mitochondria and reactive oxygen species (ROS) occur because of dysfunction of protein quality control system such as autophagy or mitophagy and proteasome. It was reported by many publications that ROS or dysfunction of autophagy were one of a cause in cellular senescence. Especially, mitophagy in dysfunctional mitochondria is important factor in senescence because of major production source of ROS.

In this presentation, we will report that liposome nanocarrier (LNC) composed of manganese porphyrin (MnP) with SOD activity and pifithrin-alpha such as p53 inhibitor suppresses cellular senescence in MSCs by inducing mitophagy. By treating LNC for senescent MSCs, the growth ability was recovered, and senescent marker such as p21 and SA-beta-Gal decreased. In addition, mitochondria membrane potential by LNC was decreased so that parkin as the marker of mitophagy was induced. We consider that the induced mitophagy eliminates dysfunctional mitochondria in senescent MSCs, was rejuvenating MSCs. We will discuss the mechanism of LNC for rejuvenation of MSCs.

Keywords

Liposome nanocarrier; Senescence; Mesenchymal stem cells

Abstract **#2108**

RESPIRATORY DYSFUNCTION INDUCED BY DUST PARTICLES IN INKJET BIOPRINTED ALVEOLAR BARRIER

Dayoon Kang$_P$[1], Sungjune Jung[2]

[1] *School of Interdisciplinary Bioscience and Bioengineering, Pohang University Of Science And Technology (POSTECH)*

[2] *Department of Material Science and Engineering, Pohang University Of Science And Technology (POSTECH)*

Corresponding author's email: kminh0630@postech.ac.kr

The need for research to analyze the effects of dust particles on the respiratory system has been highlighted because such damage causes serious respiratory problems. However, most studies of dust toxicity have been conducted in two-dimensional cell culture, animal models, and epidemiological investigations. To find out how dust can cause respiratory problems, researchers should investigate using a reliable three-dimensional structural model that mimics human nature alveoli. In this study, dust particles were applied to the previously developed three-dimensional alveoli barrier created by the inkjet bioprinting process. As a result, we observed dramatic cell apoptosis, reduced proliferation and lung dysfunction in inkjet bioprinted alveolar barriers exposed to dust particles. We also observed an increase in pro-inflammatory cytokines that stimulated the secretion of matrix metalloproteinase (MMP). To analyze the effect of increasing immune response from dust, dust was treated in dose- and time-dependent manner and alveolar tissue collapse was identified to induce structural collapse and reduced barrier robustness. We further investigated lung surfactant protein-related genes in dust-treated alveoli tissues and then estimate the harmful effects of dust on lung surfactant dysfunction. This study demonstrated the physiological effects of dust on cytotoxicity, alveolar barrier stiffness and surfactant secretion at gene expression level using inkjet bioprinted alveoli barriers. It has also been demonstrated that dust can have serious consequences that can lead to the collapse of the alveoli barrier. Using in vitro inkjet bio-printed 3D alveoli barriers, we expect this strategy to be a useful tool for identifying air pollutant exposure-related diseases.

Abstract **#2109**

MULTI-SCALE X-RAY COMPUTED TOMOGRAPHY FOR THE EVALUATION OF BONE REGENERATION IN CRITICAL-SIZED DEFECTS PROMOTED BY MAGNESIUM-BASED FIBRES

Roxane Bonithon[P][1]

[1] *Zeiss Global Centre, School of Mechanical and Design Engineering, University of Portsmouth*

Corresponding author's email: roxane.bonithon@port.ac.uk

Magnesium (Mg) alloys are of particular interest for application as degradable bone substitutes due to their appropriate mechanical properties, closer to bone than any other biomaterials (1). Once implanted, Mg implants must provide adequate mechanical support to maintain the integrity of the injured site while promoting bone ingrowth to ensure optimal tissue healing (2). The aim of this study was to employ high-resolution X-ray computed tomography (XCT) to assess the bone regeneration pattern at 4, 8 and 16 weeks following the implantation of Mg-based fibres into critical-sized defects, compared to that induced by natural bovine bone grafts (BBG) and empty controls. Mechanical characterisation was also performed on 16-weeks newly formed bone-biomaterial tissues by means of in situ XCT mechanics coupled with digital volume correlation (DVC). Mg promoted higher bone formation (0.77 ± 0.15, 0.53 ± 0.07 and 0.45± 0.06 at 16 weeks for Mg, empty and BBG, respectively) up to full restoration of critical-sized defects, with closer interaction between bone and Mg fibres, as well as adequate corrosion rate. The newly formed bone seemed to undergo mineralization (541 ± 50 mg HA.cm-3), physiological remodelling (osteocytes cavities 426 ± 221µm3) and angiogenesis after 16 weeks, enabling the Mg-bone system to gain sufficient mechanical strength (3.32 ± 0.92MPa and 152 ± 1MPa for apparent yield stress and Young's modulus, respectively). This study provides evidence that these Mg-based fibres has the ability to promote osteointegration, conduction and promotion allowing the reconstruction of critical-sized defects while maintaining the mechanical integrity of the injured site.

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Abstract #2110

EPITHELIAL-TO-MESENCHYMAL TRANSITION FOR STEMNESS CONTROL AND BIOENGINEERING STRATEGIES

Barbara Barboni[1], Valentina Russo[1], Angelo Canciello[2], Maria Rita Citeroni[3], Ying Yang[4], Nicholas Robert Forsyth[5]

[1] *University of Teramo*

2 Sbarro Health Research Organization, Philadelphia (USA)

3 Teramo University

4 Guy Hilton Research Centre, Keele University

5 Institute for Science & Technology in Medicine, Keele University

Corresponding author's email: bbarboni@unite.it

Epithelial-to-mesenchymal transition (EMT) is a key event in embryo development and post-natal life in which epithelial cells undergo a transdifferentiation into mesenchymal cells by acquiring a mobile state. This cell transitioning process recognizes the activation of signaling pathways which occur under controlled environments in response to factors leading stem cell epigenetic reprogramming, self-renew and production of differentiating progenitor lineage (1). The investigation of EMT processes controlling tissue patterning and organization as well as disregulating healing processes leading to fibrosis has been, indeed, addressed for enabling the advancement of bioengineering strategies.

In order to achieve control over the regenerative process, tissue engineering usually rely on isolation, expansion and transplantation of progenitor cells to repair target tissues (2-3). The underlying strategy is that committed progenitors will directionally support the regeneration of damaged tissue by avoiding dedifferentiation or transdifferentiation involving cellular transitions (EMT or MET) leading to adverse fate decisions or to the release of alternative factors.

Controlled EMT-mediated regenerative events are totally depend on the ability to generate favorable local environmental of stem cell-host tissue dialogue as well as repairing feedback loop between extracellular matrix (ECM) mechano-chemical signaling and progenitor cells (4).

Studies that probe the mechanisms underlying the relationship between stemness and EMT and EMT/ECM interactions thus represent the next steps forward in elucidating strategies for reducing fibrosis and selecting progenitor populations for tissue repair.

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Abstract **#2111**

AUTOMATED DEEP LEARNING CLASSIFICATION, SORTING OF ORGANOIDS, AND BIOREACTOR CULTURING OF ORGANOIDS USING FOR LIVER TISSUE ENGINEERING

Thomas Valentin[P][1], Stephanie Boder-Pasche[1], Jonas Goldowsky[1], Manon Blache[1], Christoph Netsch[1], Francesca Luongo[1], Noa Schmid[1], Baris Atakan[1], Fatemeh Navaee[1], Sarah Heub[1], Daniel Migliozzi[1], Vincent Revol[1], Gilles Weder[1]

[1] *CSEM*

Corresponding author's email: thomas.valentin@csem.ch

Liver disease is a major healthcare challenge, accounting for ~2 million deaths yearly worldwide. Liver transplantation is the best way to re-establish normal liver function and currently less than 10% of transplantation needs are met. As part of the EU Horizon 2020 OrganTrans consortium we aim to address this by developing a liver tissue printing platform for transplantation.

One critical need is a reliable source of healthy cells and liver spheroids that are used to fabricate a liver construct using bioprinting. Using robotics, microfluidics, and deep learning we have developed a novel spheroid classification and sorting platform that can image, classify, and sort 80,000 spheroids within 2 hours. This classification and sorting system is the first platform that is compatible with biosafety cabinets and any well plate design, can sort spheroids, and uses deep learning with a classification accuracy of > 98%. Images of the underrepresented unhealthy class are synthesized using generative adversarial networks for an enhanced classifier training process. The platform removes unhealthy spheroids and the healthy spheroids are then harvested and concentrated within a hydrogel precursor solution for bioprinting.

Immediately after bioprinting, the liver construct matures at physiological conditions. We are developing an automated bioreactor that enables continuous tissue perfusion via to automated fluidics while ensuring sterility. The bioreactor will incorporate sensing functions and enable the maturation of six tissues in parallel. These two research and development efforts will help to address challenges in organ engineering and begin to close the gap between patient need and transplant supply.

Abstract **#2112**

DEEP LEARNING-ENABLED AUTOMATED PROCESS CONTROL FOR HYDROGEL MANUFACTURING

Thomas Valentin[P][1], Diane Ledroit[1], Iason Sideris[1], Daniel Migliozzi[1], Sarah Heub[1], Fatemeh Navaee[1], Felix Kurth[1], Philipp Schmid[1], Gilles Weder[1], Vincent Revol[1]

[1] *CSEM*

Corresponding author's email: thomas.valentin@csem.ch

Hydrogels are a significant and growing segment of the drug delivery, tissue engineering, and wound care markets. Naturally-derived hydrogels have numerous benefits over synthetic hydrogels such as biocompatibility and biodegradability, but they often suffer from challenges such as batch-to-batch variability and sensitivity to preparation conditions such as mixing ratio, shear stress, temperature, pH, CO2 concentration, and humidity. These challenges pose a complex process control problem for high-throughput hydrogel manufacturers. In partnership with a pioneering hydrogel manufacturer, we have developed a novel process control strategy that incorporates in-line sensors and deep learning and can provide real-time process monitoring, predictive / adaptive decision-making, and prescribe corrective actions.

We have chosen collagen type I hydrogel manufacturing as a test case due to (1) our extensive expertise working with this material in industrial manufacturing settings, and (2) known end-user interest. Our strategy incorporates in-line temperature, pH, flow rate, pressure, and conductivity sensors to monitor mixing and preparation conditions. Additionally, we use environmental temperature, CO2, and humidity sensors to subsequently log crosslinking conditions. We have also developed a final optical quality control readout that will monitor the color and opacity of the final hydrogel. All of these variables are tracked in real-time against a known baseline, and a deep learning anomaly detection identifies any deviations from this baseline and can prescribe corrective actions.

Having successfully demonstrated applicability to collagen we hope to explore the application of this strategy to other biomaterial manufacturing processes such as gelatin.

Abstract **#2113**

PCL/CEL FIBROUS SCAFFOLD FOR CARTILAGE REGENERATION VIA COAXIAL ELECTROSPINNING: FROM DESIGN TO PHYSICOCHEMICAL PROPERTIES

Lauryna Dabasinskaitep[1], Edvinas Krugly[1], Odeta Baniukaitiene[1], Darius Ciuzas[1], Dainius Martuzevicius[1]
[1] *Kaunas University of Technology*
Corresponding author's email: lauryna.dabasinskaite@ktu.lt

Introduction. Poly[ε]caprolactone is one of the most suitable and desired synthetic biodegradable polymers having favourable mechanical properties and biocompatibility, however suffering from hydrophobicity. Processing of PCL by electrospinning is among the most promising routes for scaffold fabrication. Coaxial electrospinning allows the fabrication of polymer composites and blends having superior properties, such as increased hydrophilicity and controlled release of active substances.
Materials and methods. The aim of this study was to fabricate polycaprolactone (PCL)/cellulose (CEL) composite scaffold having co-axially bi-layered structure with uniform morphology and to assess the effects of attached bioactive additive on cell growth and proliferation. Cellulose acetate (CA) solution was used to fabricate shell layer of fibres, and subsequently converted to cellulose using a NaOH solution. Fibres were further coated with gelatine filled with glucosamine sulphate (GS) as an active substance to facilitate proliferation of cartilage cells. Scaffold physicochemical properties were determined by SEM, TEM, ATR-FTIR, XRD, water contact angle, chemical carboxyl group, and mechanical analysis. Biocompatibility was assessed as cell proliferation evaluated by the CCK-8 assay.
Results. Scaffold formation via coaxial electrospinning resulted in uniform PCL/CA fibers with and average diameter of ~2 µm (outer to inner ratio of 1:6). After the conversion of CA to CEL, scaffold wettability was improved, and water contact angle decreased 30-40% to ~60 degrees. The cell proliferation in the described scaffold was 20% more efficient compared to control PCL matrix without modification with CA and GS.

Keywords
coaxial electrospinning; scaffold; polycaprolactone

Abstract **#2114**

WEARABLE HUMAN SKIN GRAFTS WITH BODY-SITE-SPECIFIC PROPERTIES FOR ADVANCED SKIN REPLACEMENT THERAPY

Alberto Pappalardo[1], David E. Alvarez-Cespedes[1], Shuyang Fang[2], Abigail R. Herschman[2], Eun Y. Jeon[1], Kristin M. Myers[2], Jeffrey W. Kysar[2], Hasan Erbil Abaci[1]

[1] *Department of Dermatology, Columbia University Irving Medical Center, New York, NY 09032, USA*

[2] *Department of Mechanical Engineering, Columbia University, New York, NY 10027, USA*

Corresponding author's email: hea2113@cumc.columbia.edu

Each year, more than one million patients are hospitalized in the U.S. for significant skin loss due to injuries, diabetic ulcers or genetic skin diseases. The way we engineer skin grafts for these patients today follows the same principle that was first described nearly 30 years ago. The current approach generates skin layer-by-layer, as flat patches with open edges and disregards the fact that human skin is, in fact, a fully-enclosed organ with curved geometries. As a result, the conventional skin grafts (cSGs) typically fail to effectively cover irregular body parts, e.g., hands, due to their generic geometry and poor mechanical properties. In this study, we challenge this prevailing paradigm by reimagining skin grafts as fully-enclosed 3D tissues that recapitulate region-specific biomechanical properties of the skin and can be transplanted as a wearable tissue on any part of the body. We developed a method to generate wearable skin grafts (wSGs) to perfectly fit the recipient site. The method includes acquisition of biometric data with a laser scanner and 3D-printing a patient-specific scaffold on which wSGs are grown under continuous perfusion until they are ready for grafting. We found that wSGs have enhanced region-specific mechanical properties compared to cSGs and an improved organization of the basement membrane proteins. In addition, we generated wSGs tailored specifically to fit on mouse hindlimbs as skin sleeves and demonstrated their transplantation. Our study introduces wSGs as a compelling new technology that can make a transformative impact on the lives of patients with significant skin loss.

Abstract **#2115**

MECHANOSENSING AND MICROTISSUE REPAIR IS REGULATED BY FORCE-FAK SIGNALING COUPLING AT INDIVIDUAL FOCAL ADHESIONS

Marc A. Fernandez-Yague[P][1,2,3], Dennis Zhou[1,2,3], Ellijah Holland[1,2,3], Jeroen Eyckman[4,5], Jiamping Fu[6], Christopher Chen[4,5], David Schlaepfer[7], Andres J. Garcia[1,2,3]

[1] Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA, Atlanta, Georgia, United States of America

[2] Coulter Department of BiomedicaEngineering, Georgia Institute of Technology, Atlanta, GA 30332, USA, Atlanta, Georgia, United States of America

[3] Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA, Atlanta, Georgia, United States of America

[4] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA, Boston, United States of America

[5] Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA, Boston, United States of America

[6] Department of Mechanical Engineering, Department of Biomedical Engineering, Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA, An Arbor, Michigan, United States of America

[7] Moores Cancer Center, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Diego, La Jolla, CA 92093, USA, San Diego, California, United States of America

Corresponding author's email: marc.fdz@gmail.com

How adhesive forces are transduced and integrated into biochemical signals at focal adhesions (FAs) is poorly understood [1,2]. Using cells adhering to deformable micropillar arrays, we demonstrate that traction force and FAK localization, as well as traction force and Y397-FAK phosphorylation, are linearly coupled at individual FAs on stiff, but not soft, substrates. Similarly, FAK phosphorylation increases linearly with external forces applied to FAs using magnetic beads. This mechanosignaling coupling requires actomyosin contractility, talin-FAK binding, and full-length vinculin that binds talin and actin. Using an in vitro 3D biomimetic wound healing model, we show that force-FAK signaling coupling coordinates cell migration and tissue-scale forces to promote microtissue repair. A simple kinetic binding model of talin-FAK interactions under force can recapitulate the experimental observations. This study provides insights on how talin and vinculin convert forces into FAK signaling events regulating cell migration and tissue repair.

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Abstract **#2116**

VASCULARIZATION OF TISSUE ENGINEERED CARTILAGE - SEQUENTIAL IN VIVO MRI DISPLAY FUNCTIONAL BLOOD CIRCULATION

Peter Apelgren[P][1]

[1] *Sahlgrenska Akademin Institutionen för Kliniska Vetenskaper Plastikkirurgiska Kliniken*

Corresponding author's email: peter.apelgren@gu.se

Establishing functional circulation in bioengineered tissue after implantation is vital for the delivery of oxygen and nutrients to the cells. Native cartilage is avascular and thrives on diffusion, which in turn depends on proximity to circulation. Here, we investigate whether a gridded three-dimensional (3D) bioprinted construct would allow ingrowth of blood vessels and thus prove a functional concept for vascularization of bioengineered tissue. Twenty 10 × 10 × 3-mm 3D-bioprinted nanocellulose constructs containing human nasal chondrocytes or cell-free controls were subcutaneously implanted in 20 nude mice. Over the next 3 months, the mice were sequentially imaged with a 7T small-animal MRI system, and the diffusion and perfusion parameters were analyzed. The chondrocytes survived and proliferated, and the shape of the constructs was well preserved. The diffusion coefficient was high and well preserved over time. The perfusion and diffusion patterns shown by MRI suggested that blood vessels develop over time in the 3D bioprinted constructs; the vessels were confirmed by histology and immunohistochemistry. We conclude that 3D bioprinted tissue with a gridded structure allows ingrowth of blood vessels and has the potential to be vascularized from the host. This is an essential step to take bioengineered tissue from the bench to clinical practice.

Keywords

Vascularization; Cartilage; MRI

Abstract **#2117**

3D BIOPRINTING PHOTO-CROSSLINKABLE CARTILAGE-DERIVED EXTRACELLULAR MATRIX POROUS HYDROGEL FOR AURICULAR CARTILAGE REGENERATION

Litao Jia[P][1], Yujie Hua[2], Jinshi Zeng[1], Guangdong Zhou[2], Xia Liu[1], Haiyue Jiang[1]

[1] *Research Center of Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College*

[2] *Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine*

Corresponding author's email: litaoj@126.com

The rapid development of tissue engineering and regenerative medicine has provided a new strategy for ear reconstruction. Polyglycolic acid scaffold has been successfully used to regenerate auricular cartilage and achieved the first clinical breakthrough. However, due to acidic degradation products of polymer materials, the clinical effect was unsatisfied, which seriously limits its clinical application. Cartilage acellular matrix is one of the most potential biomimetic materials for cartilage regeneration because of cartilage matrix components and cartilage-specific microenvironment. Combined with 3D-printing, cast-molding, and freeze-drying, the mature auricular cartilage was successfully regenerated. However, pore structure, cell distribution, and three-dimensional morphology of freeze-dried scaffolds are difficult to be accurately controlled, which results in heterogeneous and unsatisfactory structure of regenerated cartilage. 3D bioprinting can realize the directional distribution of both cells and materials, and can complete the precise control of morphology, which provides a new direction to address this problem. In this study, the photo-crosslinkable cartilage-derived extracellular matrix hydrogel was successfully prepared by decellularized, enzyme digestion, and modified with methacrylic anhydride. The porous structure formed by polyethylene oxide (PEO) is beneficial to cell proliferation, migration, and extracellular matrix secretion. After imported the 3D digital model, the bioink consisted of chondrocytes and hydrogels was prepared into a human-ear-shaped construct by 3D-Bioplotter bioprinter. After photo-crosslinking and dissolving PEO, the auricular construct was transplanted subcutaneously in nude mice. Finally, the mature and homogeneous human-ear-shaped cartilage with satisfactory morphology and good elasticity was successfully regenerated, which provides scientific evidence and support for future clinical application in auricular tissue engineering.

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Abstract **#2118**

HIGHLY LOADED MICROSPHERE SCAFFOLDS FOR BONE AND CARTILAGE TISSUE ENGINEERING

Stefan Lohfeld$_P$[1], Denise Iliff[1]

[1] *University of Missouri-Kansas City*

Corresponding author's email: lohfelds@umkc.edu

Microspheres are utilized to deliver factors and drugs in tissue regeneration therapies in a controlled manner. Control mechanisms for the timely release of the substances are the material's degradation time and the shell thickness of the microspheres. Gradient tissue engineering scaffolds consisting of multiple types of microspheres can release different factors at different sites of the scaffold for an enhanced influence on tissue formation. Currently, for load bearing applications, microspheres are either in bulk scaffolds, with no defined internal architecture that may supports specific tissue formation, or are embedded in other scaffold matrix materials, where the release of encapsulated matter is also depending on the prior biodegradation of the matrix material and where only limited amounts of the factors that are to be released from the microspheres.

We created a 3D Printing bioink from PLA microspheres to fabricate scaffolds from microspheres without compromising the inherent advantages, such as the high surface-to-volume ratio of spheres and the microporosity within the struts. A subsequent sintering process with subcritical CO2 allowed for stronger scaffolds, yet preserved the microsphere characteristics. Cells and bioactive factors can be present during such sintering step.

These scaffolds allow for most effective release of factors from the microspheres. Multihead printers allow selective placement of such inks within one scaffold. Future work includes cell culturing with multigradient microsphere scaffolds, with the aim of creating scaffolds for osteochondral tissue engineering and scaffolds for large bone defects, in which the grown tissues better mimic natural tissue.

Keywords

Tissue engineering scaffold; 3D printing; microspheres

Abstract **#2119**

CANCER METASTASIS ON A CHIP

Mohammad Jouybar$_P$[1,2], Jelle J.F. Sleeboom[1,2], Andreas Pollet[1,2], Jaap M.J. den Toonder[1,2]

[1] *Microsystems, TU/e, Eindhoven, NL*

[2] *Institute for Complex Molecular Systems, TU/e, Eindhoven, NL*

Corresponding author's email: m.jouy.bar@tue.nl

Most breast cancer related deaths are not caused directly by the primary tumor, but by secondary tumors formed through metastasis to other organs [1]. Current in-vitro models rarely mimic the initial phase of metastasis: invasion. Hence, we focus on modeling breast cancer invasion and the relevant microenvironment on a chip. We develop microfluidic Cancer-on-a-Chip (CoC) devices to recapitulate essential cues in cancer microenvironment, namely (1) Extracellular Matrix (ECM) heterogeneity and (2) microvasculature. To generate the cancer niche, we use cell-embedded hydrogel encapsulation [2]. A water in oil flow-focusing device was used to encapsulate cancer cells in Matrigel beads. Next, Matrigel beads were cultured in collagen I hydrogel, mimicking the stromal ECM. This way we recapitulate the pre-invasive condition where cancer cells initially reside in a soft basement membrane before invading the fibrous and stiffer stromal ECM. Beside encapsulation method, we use alternative techniques like sugar-printing in CoC models to create the interface between two different materials. The model of ECM heterogeneity can potentially lead to better understanding of pre-invasive and invasive breast cancer.

Moreover, we use sugar-printing technology to create perfusion lumens, cast directly in ECM [3]. When seeded with endothelial cells, these form the (micro) vasculature. Combined with a neighboring channel for cancer cell culture, the process of cancer invasion, migration through ECM, and intravasation can be studied. This way we avoid using artificial materials like Polydimethylsiloxane (PDMS) which usually have drawbacks for cellular experiments.

Keywords

Cancer on Chip; Organ on Chip; Vasculature

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Abstract **#2120**

RNA SEQUENCING REVEALS MARKERS AND PATHWAYS OF T3-INDUCED CARTILAGE HYPERTROPHY

Nicoline Korthagen[P][1], Evelyn Houtman[1], Ilja Boone[1], Rodrigo Coutinho de Almeida[1], Kavitha Sivasubramaniyan[2], Rob Nelissen[3], Yolande Ramos[1], Michaela Tessari[2], Ingrid Meulenbelt[1]

[1] *Dept. Biomedical Data Sciences, Section of Molecular Epidemiology, LUMC, Leiden, The Netherlands*

[2] *Galapagos BV, Leiden, The Netherlands*

[3] *Dept. Orthopaedics, LUMC, Leiden, The Netherlands*

Corresponding author's email: n.m.korthagen@lumc.nl

Hallmark of the osteoarthritis (OA) disease pathophysiological process is the recuperation of OA chondrocytes towards a hypertrophic, growth plate-like morphology and signalling, leading to cartilage degeneration and mineralization. Previously, our group showed by genetics and functional studies that upregulation of thyroid signalling (T3) could be driving this process (1,2). Because the molecular pathways involved are poorly defined, we applied RNA-sequencing to our established ex vivo osteochondral age-related human explant model in which OA hypertrophy is triggered by T3. Hereto, full thickness osteochondral explants were isolated from the macroscopically preserved condyles of eight human knee-OA patients obtained after joint-replacement surgery. RNA-seq analysis was performed on cartilage isolated from the explants. VST normalization and DESEQ analysis was performed to identify differentially expressed (DE) genes in T3-treated versus matched untreated control samples. Pathway analysis was performed using Cluster Profiler. In total 247 significantly DE genes were identified, of which 182 genes were upregulated and 65 genes were downregulated. Next to the upregulation of some usual suspects, for example HIF-2alpha (EPAS1), we found that the most significantly up- and downregulated genes were CCDC80 and ANGPTL7, respectively. The main pathways involved were regulation of cartilage extracellular matrix and ossification. Comparison with OA GWAS literature identified 25 OA risk genes that were DE after T3 stimulation, giving new insights into the role of T3 in OA onset and progression. Our established human OA disease model of chondrocyte hypertrophy will be further exploited for preclinical studies on drug target discovery and efficacy testing.

Keywords

osteochondral; osteoarthritis; thyroid hormone

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Abstract **#2121**

INTACT CELL MASS SPECTROMETRY FOR QUALITY CONTROL OF EMBRYONIC STEM CELLS IN PRECLINICAL AND CLINICAL GRADE CULTURES

Petr Vaňhara[P][1,2], Lukáš Pečinka[1,3], Lukáš Moráň[2], Vendula Pelková[2], Hana Kotasová[1,2], Volodymyr Porokh[2], Josef Havel[1,3], Aleš Hampl[1,2]

[1] *St. Anne's University Hospital Brno*

[2] *Faculty of Medicine, Masaryk University*

[3] *Faculty of Science, Masaryk University*

Corresponding author's email: pvanhara@med.muni.cz

Human embryonic stem cells (hESCs) can differentiate into all principal cell types in human body and this capability makes the hESCs a promising tool for cell therapy, tissue engineering and variety of other bio-industrial, bio-medical and pharmaceutical applications. However, despite the currently used, stringent protocols, there is still a substantial heterogeneity in hESCs cultures that may produce aberrant cells with unwanted properties, such as lack of functional phenotype or propensity to cancer growth. Therefore, robust, easy-to-perform and sensitive methods that would determine and confirm status of cultured hESCs and also reveal divergences from optimal differentiation trajectory are needed. Here we modelled some typical unwanted scenarios that may occur in long-term cultures of hESCs. These involve development of karyotype aberrations, culture cross-contamination, and failure of hESCs to differentiate into a functional phenotype. The cultures of hESCs and their differentiation stages were analyzed by intact cell mass spectrometry followed by advanced statistical analysis of the mass spectra. Spectral fingerprints reflecting the chemical composition of the inner cellular environment contained sufficient information to identify normal and aberrant hESCs, or to confirm correct differentiation trajectory by cluster analysis and machine learning approaches.

In summary, intact-cell mass spectrometry is a promising tool for quality control in long-term preclinical and clinical stem cells cultures. This study was supported by funds from Ministry of Health of the Czech Republic (project: NV18-08-00299) and from Faculty of Medicine of Masaryk University (project: MUNI/A/1689/2020).

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Abstract **#2122**

VISUALIZATION OF COLLAGEN FIBRE STRUCTURE IN OSTEOGENESIS IMPERFECTA USING SECOND HARMONIC GENERATION IMAGING ON POLYCAPROLACTONE SCAFFOLD

Tugba Cebe$_P$[1,2], Meena Balasubramanian[3], Nicola Green[2], Gwendolen Reilly[1,2]

[1] *Insigneo Institute for in silico Medicine University of Sheffield, Sheffield, S3 7HQ*

[2] *The Kroto Research Institute, Department of Materials Science and Engineering, University of Sheffield, Sheffield, S3 7HQ*

[3] *Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, S5 7AU*

Corresponding author's email: tcebe1@sheffield.ac.uk

Osteogenesis Imperfecta (OI) is a major connective disorder caused by type I collagen (Col I) mutations [1].To date much OI research has been performed as an ex vivo technique. Second Harmonic Generation (SHG) has recently been used to visualize the extracellular matrix (ECM) of OI tissues[2]. This project aims to create an in vitro, human-cell based model of ECM deposition using Polycaprolactone (PCL) scaffolds to understand how Col I mutations alter the early collagen formation processes in 3D. PCL mats were fabricated by electrospinning as non-aligned and aligned fibers. Primary Human Dermal Fibroblasts (HDF) were collected from human donors with OI under informed consent from Sheffield's Children Hospital and healthy HDFs (adult) from PromoCell. Cell-secreted collagen was analyzed using a laser scanning confocal microscope fitted with a Ti: sapphire laser. The results indicated that collagen secreted by healthy HDFs aligned in the direction of the electrospun PCL fibres [3].Notably, collagen secreted by OI fibroblasts cultured on aligned and nonaligned fibres also produced SHG signals. The SHG signal produced by OI fibroblasts on aligned scaffolds are stronger than nonaligned scaffolds, although the collagen produced by OI HDF is less ordered. Sirius Red Assay supported the indication that OI HDF can secrete more collagen on aligned substrates. Our results demonstrate that fibrous scaffolds can be used to create in vitro human cell-based models in 3D. This will be a tool to better understand the mechanisms behind diseases of collagen such as OI. Acknowledgement: Republic-of-Turkey-Ministry-of-National Education. H2020 – MSCA – RISE - 2017(777926).

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Abstract **#2123**

ELECTROSPUN EXTRACELLULAR MATRIX SCAFFOLDS FOR NEURAL REGENERATION

Lena Mungenast$_P$[1]

[1] *FHNW*

Corresponding author's email: lena.mungenast@fhnw.ch

Decellularization of tissues, with the goal of applying extracellular matrices (ECM) based scaf-folds in regenerative medicine, requires the preservation of the complex biochemical cues specif-ic to the native microenvironment (1-4).
In this work, cell-free, spinal cord-derived ECM scaffolds were produced to support the alignment of neural progenitor cells and neurons. Decellularization of pig spinal cord was accomplished by a combination of mechanical, physical (freeze-thawing) and chemical (SDS incubation) treat-ments. The decellularized tissue was characterised via dsDNA quantification and histological staining to assess the presence of cell nuclei, proteins and glycosaminoglycans. Successfully decellularized spinal cord was lyophilized, milled and solubilised into a bioink, using hexafluoroi-sopropanol (5 % w/v ECM + 0.1 % w/v Polyethyleneglycol) and patterned into an electrospun fiber scaffold (using a 3D Bioprinter). The results show that porcine spinal cord can be success-fully decellularized leading to an ECM with less than 50 ng/mg dsDNA, and without cell nuclei. The formulated bioink enabled spinning of acellular spinal cord ECM scaffold with fibers of <10 µm diameter. The non-cytotoxic scaffolds supported the viability of a human neural cell line (SH-SY5Y) over the course of 14 days and the selective differentiation into neurons, as confirmed by immunolabeling of specific cell markers (HB9, Tubulin ß). The combination of biochemical and topographical cues in this patterned ECM-derived scaffold induced the alignment of neural cells in vitro. The strategy of combining decellularized tissues followed by specific deposition patterns to opti-mize biochemical and topographical cues opens the way for clinically relevant central nervous system scaffolding solutions.

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Abstract **#2124**

BIOACTIVE 3D SCAFFOLDS FOR THE DELIVERY OF NEUROTROPHINS TO IMPROVE NEURITE OUTGROWTH AND INDUCE SCHWANN CELL MIGRATION

Ana M Sandoval-Castellanos[P][1], John Haycock[1], Frederik Claeyssens[1]

[1] *University of Sheffield*

Corresponding author's email: amsandovalcastellanos1@sheffield.ac.uk

Peripheral nerve injury causes disability [1-2]. The clinical standard is the use of autografts, however, their performance is limited due to donor site morbidity. The addition of neurotrophins has been studied to improve the performance of nerve guide conduits (NGCs), however, nerve regeneration is still not optimal [3-4]. Therefore, it is paramount to design a platform for the sustained delivery of neurotrophins to provide a continual stimulation to axonal outgrowth and nerve regeneration [5]. The aim of this study was to fabricate a novel bioactive surface on electrospun fibers to provide a sustained release of heparin-bound NGF or BDNF to stimulate neurite outgrowth and Schwann cell (SC) migration [6]. The bioactive surface was characterized by XPS analysis and ELISA. To study the effect of the bioactive 3D scaffold, nerve regeneration was evaluated with primary nerve tissue, specifically, DRGs explants were seeded on the scaffolds, and axon outgrowth and SC migration were measured. Our results showed significantly enhanced nerve outgrowth, with the formation of neurites of up to 3 mm, accompanied by SC. We hypothesize the combination of physical guidance provided by the fibres along the sustained delivery of relevant biological factors, NGF and BDNF, provided a stimulatory environment for nerve regeneration [7-8], where the delivery of neurotrophins was sustained for 21 days. Importantly, our results were achieved by immobilizing relatively low concentrations of neurotrophins (1 ng/mL), which provides a promising, low-cost and scalable method to improve current NGCs. Future studies will assess the performance of the bioactive scaffold in vivo.

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Abstract **#2125**

3D IN VITRO MODELLING OF OVARIAN CANCER BY MEANS OF ADDITIVE MANUFACTURED CHITOSAN-BASED POLYELECTROLYTE COMPLEXES

Dario Puppi[1], Simona Braccini[P1], Gianni Pecorini[1], Chiara Tacchini[1], Federica Chiellini[1]

[1] *Department of Chemistry and Industrial Chemistry, University of Pisa*

Corresponding author's email: simona.braccini@phd.unipi.it

Polyelectrolyte complexes (PEC)s are supramolecular self-assembled systems formed upon combination of oppositely charged compounds by means of electrostatic interactions [1]. The possibility of directly mixing polyelectrolytes of natural origin with inherent biocompatibility, to obtain PECs with controlled water stability, biodegradability, and mechanical properties, has attracted great interest for various biomedical applications. In particular, additive manufacturing (AM) integration with naturally-derived PEC strategies is resulting into novel layered hydrogels designed for engineering human tissues [2]. This research activity's aim was the development of additive manufactured PECs based on chitosan, the reference cationic polyelectrolyte from natural source, and designed for 3D in vitro modelling of ovarian cancer. To this purpose, either alginate or hyaluronic acid were investigated as polyanions to form PEC aqueous suspensions suitable to be processed by AM. Experimental fabrication protocols were optimized to process suspensions with different polycation/polyanion weight ratio into 3D porous hydrogels through direct extrusion into an ethanol bath. The influence of PEC composition on hydrogel's physical-chemical and mechanical properties was clearly demonstrated. Structural stability in cell culture medium of optimized PEC hydrogel prototypes was exploited for in vitro tumor tissue growth. In vitro biological characterization highlighted how the adhesion, morphology, proliferation, and migration of A2780 ovarian cancer cell line kept in culture for periods up to 3 months, were significantly affected by PEC composition. Ongoing experimental investigations are dedicated to evaluating in vitro chemotherapeutics response of the 3D tissue models as well as to assess cisplatin-resistant A2780cis cell line cultured on the developed PECs.

Keywords

Polyelectrolyte Complexes; Ovarian Cancer; Solution/Suspension Additive Manufacturing

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Abstract **#2127**

DEVELOPMENT OF LUNG CELL MODEL FOR CLINICAL AND ENVIRONMENTAL INVESTIGATIONS

Aleš Hampl[P][1,2], Hana Kotasova[1,2], Vendula Pelkova[2], Michaela Capandova[2], Zuzana Koledova[2], Lukas Moran[2], Martina Karasova[3], Jan Vondracek[3], Lukas Pecinka[4,1], Josef Havel[4,1], Petr Vanhara[1,2]

[1] *St. Anne's University Hospital, Brno, Czech Republic*

[2] *Faculty of Medicine, Masaryk University, Brno, Czech Republic*

[3] *Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic*

[4] *Faculty of Science, Masaryk University, Brno, Czech Republic*

Corresponding author's email: ahampl@med.muni.cz

Human respiratory system is constantly being exposed to outside environmental factors. Continuous exposure to toxic and/or infectious agents can cause many airway diseases such as asthma, chronic obstructive pulmonary disease, obliterative bronchiolitis, and cystic fibrosis. One of the most serious environmental issues are the air pollutants. Many of the toxic effect of air pollution have been linked with polycyclic aromatic hydrocarbons (PAH). PAH are ligands of the aryl hydrocarbon receptor (AhR) that has participated in detoxification and has been also shown in immune response within the lung tissue. Nevertheless, numerous others aspect of AhR signaling within the lung epithelium still remain unclear.
To investigate the role of AhR in detoxification and biodegradation of air pollutants we are developing cell model of normal lung epithelium. We generated the cells differentiated in vitro from human embryonic stem cells (hESC) that can be propagated for long-term in culture and most likely represent equivalent of expandable lung-like epithelia (ELEP). The hESC-derived ELEP express markers of early lung epithelial lineage and display properties of cells in early stages of surfactant production. They have a high proliferative potential in vitro and are capable of differentiation into mature epithelial cells. Under 3D culture conditions, both in vitro and in vivo, ELEP arrange themselves into structures resembling normal lung tissue. To investigate the involvement of AhR in lung epithelia formation, functioning, and detoxification, we have recently developed ELEP with knocked-out AhR gene.

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Abstract **#2128**

HYDROXYTYROSOL, A FEASIBLE EFFECTIVE COMPOUND IN COMBATING INTIMAL HYPERPLASIA FORMATION EX VIVO: AN IN VITRO PRELIMINARY STUDY

Ubashini Vijakumaran[P1], Muhammad Dain Yazid[1], Mohd Ramzisham Abdul Rahman[2], Nadiah Sulaiman[1]

[1] *Center of Tissue Engineering and Regenerative Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia*

[2] *Department of Surgery, Hospital Canselor Tuanku Mukhriz, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia*

Corresponding author's email: ubashini.shini@gmail.com

Background: Intimal hyperplasia (IH) is a response to endothelial cell (EC) injury which triggers uncontrolled proliferation of smooth muscle cells (SMC). IH decreases the patency of bypass graft post Coronary artery bypass graft surgery (CABG) and threatens angioplasty procedure. Antiproliferative drugs such as paclitaxel and sirolimus were inhibited IH but also impede the re-endothelisation. Hydroxytyrosol is an olive-derived polyphenol. Its effect on IH attenuation has not been extensively studied. Therefore, we aimed to study the effect of HT in EC proliferation and SMC inhibition in-vitro before its application in an ex-vivo IH model.

Methods: EC and SMC were collected from saphenous veins of patients undergoing CABG surgery at Hospital Canselor Tuanku Mukhriz (HCTM), Malaysia. Dose response curve plotted using MTT assay. Whereas HT effect on proliferation and apoptosis were examined using EdU Cell Proliferation and TUNEL Assay. VEGFR2 expression evaluated via western blotting.

Results: We found 80uM of HT significantly increases the proliferation of EC and decreases apoptosis in EC. The maximal effective concentration (EC50) is 78.1uM ($p<0.05$). VEGFR2 protein which responsible for angiogenesis was highly expressed in HT-treated EC. The half-maximal inhibitory concentration (IC50) of HT-treated SMC is 300uM. A concentration of 80uM up to 320uM of HT potentially decreases the proliferation of SMC by inducing apoptosis.

Conclusions: HT boosts EC proliferation whilst inhibit SMC proliferation in-vitro in a concentration-dependent manner. Therefore, these preliminary findings enable the study of HT combinatory effect on both EC and SMC in an ex-vivo IH model to be elucidated in the future.

Keywords

Hydroxytyrosol; Intimal Hyperplasia; Endothelial cells

Abstract **#2129**

EXTRACTION OF MATRIX-BOUND NANOVESICLES (MBVS) FROM HIGH-HYDROSTATIC PRESSURE DECELLULARIZED TISSUE AND EVALUATION ON VASCULAR ENDOTHELIAL CELLS

Mako Kobayashi[P][1], Naoki Ishida[1], Yoshihide Hashimoto[1], Jun Negishi[1], Hideki Saga[2], Yoshihiro Sasaki[3], Kazunari Akiyoshi[3], Tsuyoshi Kimura[1], Akio Kishida[1]

[1] *Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University*

[2] *KM biologics Co., Ltd.*

[3] *Kyoto University*

Corresponding author's email: makombme@tmd.ac.jp

Decellularized tissues are those in which extracellular matrix (ECM) – derived scaffolds are widely used as a promising material for tissue engineering and regenerative medicine. As for the mechanism of biocompatibility and functionality of decellularized tissue, several exploratory researches have been studied focusing on the ECM structure and components. Recently, the presence of matrix-bound nanovesicles (MBVs) embedded within the ECM has been reported 1), but the details are still unclear. We have previously reported that decellularized tissues prepared by high hydrostatic pressure (HHP) method show good in vivo performance 2, 3). In the present study, we focused on the MBVs and investigated the extraction method of MBVs from HHP decellularized small intestinal submucosa (SIS) and their effects on vascular endothelial cells. H-E staining and DNA quantification showed that the nuclei were removed in HHP treated SIS. With nanoparticle tracking assay and transmission electron microscope (TEM) observation, nanosized (50-200 nm) and membranous particles similar to MBVs were detected. To evaluate the effect on angiogenesis, which is important in the process of tissue reconstruction, vascular endothelial cells were exposed to isolated MBVs and cell proliferation was evaluated. Isolated MBVs-like vesicles induced endothelial cell proliferation. These results suggested that MBVs-like vesicles can be extracted from HHP decellularized tissue and have important role in angiogenesis.

Keywords

nanovesicles; vascular endothelial cell

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Abstract **#2130**

ZWITTERIONIC POLYMER-DRUG CONJUGATES WITH LUBRICATING, CHONDROPROTECTIVE ACTIVITY FOR THE TREATMENT OF OSTEOARTHRITIS

Patrick Weber[P][1], František Surman[1], Maryam Asadikorayem[1], Marcy Zenobi-Wong[1]

[1] *Tissue Engineering and Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zurich, CH-8093 Zurich, Switzerland*

Corresponding author's email: patrick.weber@hest.ethz.ch

Osteoarthritis (OA) is the leading cause of joint disability worldwide. Despite the growing disease burden, no disease-modifying formulation has so far entered the clinic with limited control over the spatiotemporal drug release profile being one of the main challenges[1]. Here, we report on a novel polymer-drug conjugate with specificity towards cartilage tissue, controlled release of a chondroprotective drug and lubricating activity. The polymeric backbone of the system consists of a poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) chain. Zwitterionic polymers such as PMPC have long been recognized for their excellent biocompatibility and non-fouling properties[2,3]. In addition, their biological equivalents – phosphatidylcholine lipids – are known to play an important role in joint lubrication which has inspired recent research on synthetic PMPCs to improve lubrication of defective cartilage and slow OA disease progression[4,5]. We found that these polymers penetrate well into cartilage explants and we were able to achieve greater than 80% retention by conjugating them to the cartilage-specific, collagen II-binding hexapeptide WYRGRL. Regarding the therapeutic component of the system, we performed experiments with the IκB kinase inhibitor BMS-345541[6] on chondrocytes co-treated with the inflammatory cytokine IL-1β and found near complete restoration of the untreated phenotype in 2D culture. By conjugating the drug to our zwitterionic backbone through a matrix metalloproteinase-cleavable linker a triple-functional – targeted, chondroprotective and lubricating – drug depot for the disease-modifying treatment of osteoarthritis was designed.

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Abstract **#2131**

DEVELOPMENT OF HUMANIZED 3D KIDNEY TISSUE MODELS FROM DECELLULARIZED RAT PRECISION-CUT KIDNEY SLICES

Haitham Salti[P][1], Lea Kramer[1], Sophie-Charlotte Nelz[1], Mathias Lorenz[2], Daniela Schwerdt[2], Steffen Mitzner[1,3], Reinhold Wasserkort[1,3]

[1] *Fraunhofer Institute for Cell Therapy and Immunology (IZI), Department of Extracorporeal Therapy Systems (EXTHER), Rostock, Germany*

[2] *Hochschule Wismar, Faculty of Engineering, Wismar, Germany*

[3] *Rostock University Medical Center, Department of Internal Medicine, Nephrology, Rostock, Germany*

Corresponding author's email: haitham.salti@izi.fraunhofer.de

The kidney is a complex organ that includes around 30 cell types, of which renal proximal tubule cells (RPTEC) represent 43% [1,2]. This project aims at developing 3D kidney tissue models for the investigation of recellularization strategies to support developing tissue-engineered kidneys. Decellularization of precision-cut kidney slices (PCKS) represents a promising source of scaffolds. However, effective recellularization depends largely on the decellularization method. While decellularization of PCKS by immersion in chemicals is a standard approach, it is necessary to increase scaffolds quality. We explored the potential of reducing contact time with chemicals by treating PCKS physically before immersion, with high hydrostatic pressure (HHP) or repeated freezing-thawing cycles (FTC).

Rat PCKS were decellularized using two approaches. First: immersion of PCKS in chemicals. Second: physical treatment combined with immersion in chemicals. The combined approach consists of 3 different protocols: 1) sonication during immersion 2) HHP treatment before immersion 3) FTC treatment before immersion. Decellularization of PCKS by immersion followed a slightly modified protocol: 0.025% trypsin, 2% Triton X-100, 2% SDC and 0.1% SDS [3]. Data revealed that the combined chemical and physical approach is more effective in retaining GAGs (glycosaminoglycans) than immersion alone. While HHP retained more GAGs than ultrasound, both showed a higher GAG content than decellularization by immersion alone. However, HHP was less efficient in DNA removal than FTC. Moreover, histological analysis indicated that FTC is better in preserving the ECM. Currently, differently decellularized scaffolds are being used for recellularization with human RPTEC/TERT1 cells and will be assessed microscopically.

Keywords

Tissue models; Precision-cut kidney slices ; Decellularization

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Abstract **#2132**

GLYCINE-POLY(ESTER AMIDE)S FIBER FOR PROMOTING ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

Hongjuan Weng[P][1,2], Lorenzo Moroni[1], Katrien V. Bernaerts[2]

[1] *Complex Tissue Regeneration Department, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University*

[2] *Aachen-Maastricht Institute for Biobased Materials (AMIBM), Maastricht University*

Corresponding author's email: h.weng@maastrichtuniversity.nl

Damage to anterior cruciate ligament (ACL) is one of the most frequently occurring joint dysfunctions in the clinic. Due to the poor self-healing capacity, the ACL repair is a considerable challenge. It is a promising treatment to reconstruct ACL by mimicking the composite and structure of ACL via tissue engineering methods. ACLs are mainly composed of highly aligned and stiff collagen fibers surrounded by the extracellular matrix (ECM)[1].

Amino acid based poly(ester amide)s (AA-PEAs) are potential materials to simulate ACL fiber. Because PEAs combine the good mechanical, thermal and processing properties of polyamides, with the degradability of polyesters into a single system [2]. Besides, amino acids impart chemical functionality allowing further polymer modification, as well as biological functionality like cell adhesion and enzymatic degradation [3]. Specially, glycine is the most abundant amino acid in collagen, with excellent biocompatibility.

Therefore, semicrystalline glycine-PEAs with different molecular weights were synthesized by active solution polycondensation. These glycine-PEAs were thermally stable with glass temperature and melting point around 22oC and 168oC, respectively. These 3D-printed glycine-PEAs fibers were further characterized mechanically to investigate their potential use for ACL regeneration.

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Abstract **#2134**

EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELLS COMBINED WITH TISSUE ENGINEERING FOR MYOCARDIAL REPAIR IN A PIG MODEL OF MYOCARDIAL INFARCTION

Marta Monguió-Tortajada[P1,2], Cristina Prat-Vidal[1,2,3], Daina Martínez-Falguera[1,2,4], Micaela Munizaga-Larroudé[1,2,5], Carolina Soler-Botija[1,2], Miriam Moron-Font[6], Adriana Cserkoova[1], Antoni Bayes-Genis[1,2,7,8], Francesc E. Borràs[6,9], Santiago Roura[1,2,10], Carolina Gálvez-Montón[1,2]

[1] ICREC Research Program, Health Science Research Institute Germans Trias i Pujol (IGTP), Can Ruti Campus, Badalona, Spain

[2] CIBERCV, Instituto de Salud Carlos III, Madrid, Spain

[3] Institut d'Investigació Biomèdica de Bellvitge-IDIBELL, L´Hospitalet de Llobregat, Spain

[4] Department of Medicine, Universitat de Barcelona (UB), Barcelona, Spain

[5] Department of Medicine, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

[6] REMAR-IVECAT Group, Health Science Research Institute Germans Trias i Pujol (IGTP), Can Ruti Campus, Badalona, Spain

[7] Cardiology Service, Germans Trias i Pujol University Hospital, Badalona, Spain

[8] Department of Medicine, UAB, Barcelona, Spain

[9] Nephrology Service, Germans Trias i Pujol University Hospital, Badalona, Spain

[10] Faculty of Medicine, University of Vic-Central University of Catalonia (UVic-UCC), Vic, Barcelona 08500, Spain

Corresponding author's email: mmonguio@igtp.cat

Introduction: Accumulating evidence supports the potential of extracellular vesicles (EVs) from mesenchymal stromal cell (MSC) as therapy for cardiac healing after myocardial infarction (MI). Nevertheless, neither their efficient administration nor their therapeutic mechanisms are fully elucidated.

Purpose: To evaluate the preclinical efficacy of a tissue engineering approach to locally deliver porcine cardiac adipose tissue MSCs-derived EV (cATMSC-EV) in an acute MI pig model.

Methods: Pigs (n=24) were blindly randomized after 30 min of MI induction to an Untreated group or treated with the implantation of a tissue engineered graft composed of a decellularized human pericardial scaffold filled with peptide hydrogel and either cATMSC-EV purified by size exclusion chromatography (EV-Treated group) or buffer (Control group), placed over the post-MI myocardium. Cardiac troponin levels and MRI revealed consistent myocardial damage and infarct size amongst groups.

Results: After 30 days, cardiac function was significantly improved by graft implantation, with maintained left ventricle ejection fraction and increased aortic flow flux. cATMSC-EV delivery improved right ventricle ejection fraction (p=0.026), prevented myocardial adverse remodelling and decreased distal fibrosis (p=0.030). cATMSC-EV-treated animals had a reduced scar size (p=0.042), concomitant with increased vascularization in the infarct core (p=0.019), less macrophage infiltration (p=0.026) and more with anti-inflammatory phenotype (%CD73+; p=0.015). Surprisingly, local delivery of cATMSC-EV also triggered a systemic effect, reducing leukocyte mobilization post-MI and modulating systemic CD73+ and CCR2+ monocytes, related to immunomodulation and fibrosis modulation.

Conclusions: These results highlight the clinical potential of cATMSC-EV in modulating key features of ischemic injury and promoting cardiac repair after MI.

Keywords

Myocardial infarction; Cardiac scaffold; Pig model

Abstract **#2135**

HIGHLY ELASTIC SCAFFOLDS PRODUCED BY MELT ELECTROWRITING

Raquel Sanchez Diaz$_P$[1]

[1] *Queensland University of Technology*

Corresponding author's email: sanchezdiazraquel@gmail.com

Melt electrowriting (MEW) is an additive manufacturing technique with the ability to create micro- to nano-fibers and collect them in a well-defined layered structure, a process highly advantageous in tissue engineering applications.[1] There is a limited selection of polymers that can be melt electrowritten. Poly(ε-caprolactone) (PCL), with exceptional thermal properties, is the gold standard for MEW.[2] The alternative polymers often require tailored synthesis or high processing temperatures that cause degradation.[3]

In this study, we introduce a rapid and efficient method to adapt commercially available polymers for MEW. We use a controlled thermal pre-treatment to tailor the melt viscosity. An elastomeric commercial copolymer, poly(L-lactide-co-ε-caprolactone) (PLCL), was subjected to this pre-treatment and successfully melt electrowritten at low temperature for the first time. The elasticity of PLCL was maintained during the process, where scaffolds showed negligible deformation after cyclic extensions up to 50% of strain. PCL cannot currently deliver this type of elastic mechanic performance without printing complex architectures.

This overall approach can be readily adopted by any MEW user and is also generally suitable for thermally degrading polymers beyond MEW, where there is a need to reduce thermal degradation during processing.

Keywords

Melt Electrowriting; Thermal degradation; Polyester

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Abstract **#2136**

THE EFFECT OF NANOHYDROXYAPATITE INCOPERATED WITH MICRO RNA 21 TO REGULATE OSTEOGENESIS

Revatyambigai Subramaniam[P1], Ikram Alias[2], Jia Xian Law[1], Min Hwei Ng[1]

[1] Centre for Tissue Engineering and Regenerative Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Cheras 56000, Kuala Lumpur, Malaysia

[2] Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Cheras 56000, Kuala Lumpur, Malaysia

Corresponding author's email: regakutty2020@hotmail.com

Background: Bone is highly specialized connective tissue with unique property in bone regeneration. Hydroxyapatite due to its bioactivity has been used in osseous defect to improve osteointegration. Micro RNA 21 is a biomolecule endogenously expressed to regulate osteogenesis. Coupling of miR-21 and nHA is a potential intervention to harness greater and rapid healing of the host.

Methods: Wharton's Jelly cells obtained from umbilical cord were osteoinduced then treated with nHA and miR 21. The size and morphology of the nHA was characterized by (DLS) and (FESEM). Dose curve response and proliferation rate for the hWJMSCs treated with nHA and miR 21 was assessed by using Presto Blue assay. The expression of bone proteins was evaluated via Western Blotting. The expression of miR 21 with FAM tagged were identified by confocal imaging.

Results:
Both the FESEM and DLS showed the nano range of the nHA (10-100nm) and in spherical morphology. The dose curve response demonstrated the range of nHA 50ug/ml between 250ug/ml shows no toxicity and increased proliferation rate. The concentration of 20nM to 50nM of miR 21 evaluated show no toxicity. The western blots results expressed bone related proteins. The con-focal imaging shows the tagged miR 21 has been readily taken up by the cells.

Conclusion: hWJMSCs cells shows no toxicity after treated with nHA and miR 21 and also expresses significantly higher bone proteins. This preliminary study findings will be furthered with in vivo study to prove nHA incorporated with miR 21 will accelerate the new bone formation.

Abstract **#2137**

BIOCOMPATIBILITY OF RH-BMP-2-LOADED MESOPOROUS SILICA WITH PERIODONTAL LIGAMENT CELLS

Eleana Kontonasaki[P][1], Dimitrios Gkiliopoulos[2], Ioannis Tsamesidis[1], Anna Theocharidou[1], Konstantinos Triantafyllidis[2]

[1] *Department of Dentistry, Aristotle University of Thessaloniki*

[2] *Department of Chemistry, Aristotle University of Thessaloniki*

Corresponding author's email: kont@dent.auth.gr

A promising way to control peri-implantitits, a bacteria-associated disease causing bone loss around dental implants, is the local administration of antimicrobial drugs or growth factors at the implant site. Recombinant human bone morphogenetic protein-2 (rh-BMP-2) can effectively promote bone regeneration and osseointegration [1]. Mesoporous materials constitute promising carriers for the delivery of various cargos and could be implemented in the local administration of rh-BMP-2. The aim of the present study was to evaluate the hemolytic properties of novel, rhBMP-2-loaded mesoporous silica and their effect on the viability of human periodontal ligament cells (hPDLCs). Mesoporous silica was synthesized via sol-gel reaction, following the co-operative self-assembly route in acidic media. Tetraethyl orthosilicate (TEOS) was used as the silica source and Pluronic P123 as the structure directing agent. The loading of rh-BMP-2 was obtained after immersion in PBS, stirring and subsequent centrifugation. The effect of rh-BMP-2-loaded mesoporous silica on cell viability was evaluated through indirect MTT test. The hemolytic activity was tested at various concentrations (12.5-500μg/ml) in contact with human red blood cells. At all concentrations low or no hemolytic activity was recorded. A higher hPDLCs proliferation rate was recorded after loading with rhBMP-2. These findings suggest the potential use of the synthesized mesoporous silica as biocompatible and effective carrier for the local delivery of rhBMP-2 in peri-implantitis treatment strategies.

Acknowledgments

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Keywords

mesoporous silica; rh-BMP2; periodontal ligament cells

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Abstract **#2138**

A NOVEL PROTEIN ENGINEERING TOOL TO INFLUENCE COVALENT COUPLING

Viktoria Niklaus$_P$[1], Joachim Nickel[2], Jörg Teßmar[1], Jürgen Groll[1]

[1] *Department for functional materials in medicine and dentistry University of Wuerzburg, Germany*

[2] *Department of Tissue Engineering and Regenerative Medicine, University Hospital Wuerzburg Germany*

Corresponding author's email: viktoria.niklaus@fmz.uni-wuerzburg.de

In the last decay, recombinant protein-based therapies have shown great potential and achieved successful results in clinical applications. (1).

The major challenges rely on the optimization of existing delivery strategies since the required high protein concentrations are extremely cost-intensive. Furthermore, high unphysiological dosages must be prevented to avoid severe side effects. Therefore, innovative delivery techniques are needed in order to improve treatment efficacy with a simultaneous reduction of the protein being applied thus reducing costs and side effects. An immobilization of the bioactive proteins onto appropriate scaffold appears here to be a sound strategy to establish such next-generation delivery systems.

But how to maintain high protein activity and achieve reproducible coupling efficacy? To guarantee a high bioactivity a site-specific coupling mechanism is essential. Therefore, we adapted a tunable and novel coupling technique on the protein level (2). The required tools are embedded within the recombinant protein and on the surface of the corresponding scaffold structures. Already upon protein expression modifications are introduced within the protein sequence thereby maintaining a high protein bioactivity.

After protein purification the resulting protein can be covalently coupled to scaffolds being composed of polyoxazolines which already showed good biocompatibility and therefore are suitable for biomedical applications (3).

Keywords

Synthetic polymers; Drug Delivery; Biomaterials

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Abstract **#2139**

ESTABLISHMENT OF FUNCTIONAL CELL-BASED TEST SYSTEMS OF SWEAT GLANDS AND PAROTID GLANDS

Leonie Hilger[P1], Teresa Buck[2], Anna Emilia Matthießen[2], Heiko Benzin[1], Jan Hagemann[3], Torsten Fauth[4], Charli Kruse[1,2], Matthias Brandenburger[1]

[1] Fraunhofer Research and Development Center for Marine and Cellular Biotechnology EMB, Lübeck, Germany

[2] University of Lübeck, Institute for Medical and Marine Biotechnology IMMB, Lübeck, Germany

[3] University of Mainz, School of Medicine Department of Otolaryngology, Head and Neck Surgery, Mainz, Germany

[4] BRAIN Biotech AG, Zwingenberg, Germany

Corresponding author's email: leonie.hilger@emb.fraunhofer.de

Sweat glands and salivary glands share a similar secretion mechanism. The secretion is based on ion and water currents towards the glandular lumen which are triggered by Ca2+ signaling following adrenergic or cholinergic stimulation[1,2]. Both glands fulfill crucial physiological functions (temperature homeostasis, digestion). However, disturbed glandular activity caused by disease or therapeutical side effects often leads to disorders such as hyperhidrosis or xerostomia. Currently available cell lines often lack functional markers which are required for activation of sweat and saliva formation via Ca2+ signaling and the exploration of bioactive substances. Therefore, ongoing research strives to assemble improved functional models[3-5]. In this study, new protocols for the isolation of functional glandular cells were established. In addition, it was analyzed whether three-dimensional cultivation could enhance the expression of functional markers.
First, explant cultures of epithelial sweat gland and parotid gland cells and parotid gland-derived stem cells (PDSCs) were generated. Their expression profile was characterized by qRT-PCR and acetylcholine-elicited Ca2+ signaling in epithelial cells was analyzed with Fluo-4/FuraRed by laser scanning microscopy. In Ca2+ imaging epithelial sweat gland cells revealed acetylcholine excitability and excitation dynamics on a cellular level.
To further enhance functional gene expression, PDSCs were cultivated in agarose microwells. With this technique large numbers of PDSC spheroids could easily be generated. Further studies will reveal whether three-dimensional cultivation enhances in vitro functionality.
Advancements of these models will allow testing of bioactive substances in a more physiological context and hence enabling the development of new products for antiperspiration and restoration of salivation.

Keywords

Glandular test systems; Organoids; Primary cell lines

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Abstract **#2140**

RE-DIFFERENTIATION OF IN VITRO DEDIFFERENTIATED OVINE CHONDROCYTES: A COMPARATIVE STUDY BETWEEN HYDROGELS AND CRYOGELS

Tosca Roncadaᵽ[1], Gordon Blunn[1], Marta Roldo[1]

[1] *University of Portsmouth, School of Pharmacy and Biomedical Sciences, St Michael's Building, White Swan Road,Portsmouth, PO1 2DT, UK*

Corresponding author's email: tosca.roncada@port.ac.uk

Focal cartilage defects are a common knee problem with a high prevalence (20%) among people of all ages [1]. Due to its avascular nature cartilage has limited ability to self-repair and defects can lead to pain and to osteoarthritis in the long term. To repair these defects, autologous chondrocyte implantation (ACI) has been used for two decades with successful surgical outcomes, however, one of its main limitations is chondrocyte (CHs) de-differentiation during in vitro expansion [2]. Here we isolated ovine CHs and studied their de-differentiation from P1 to P4 in 2D culture. We then encapsulated dedifferentiated CHs into collagen and alginate (col/alg) hydrogels and cryogels and hypothesised that these 3D scaffolds would support the cells re-differentiation to CHs in the absence of chondrogenic inducers. From P1 to P4, CHs expanded in 2D culture lose their rounded morphology (aspect ratio of 1.5) to acquire a spindle shape with abundant stress fibres; they present progressive loss of chondrogenic markers (SOX9, SOX5, SOX6, COL2A1); and over-express hypertrophic markers (COL1A1). Dedifferentiated CHs embedded into col/alg hydrogels and cryogels remained viable up to day 14; furthermore, they started to aggregate and regain the typical rounded morphology and produce aggrecan and Coll II. Gene expression analysis revealed an increase of chondrogenic markers (SOX9, FOXO1, FOXO3A, COL2A1) in both 3D cultures suggesting that both formulations support the re-differentiation of in-vitro expanded CHs potentially enhancing the success rate of ACI procedures.

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Abstract **#2141**

POROUS POLYMER SCAFFOLDS AS MATRICES FOR IN VITRO TISSUE MODELS

Ahmed Eissa[1], Emma Salisbury$_P$[2]

[1] *University of Warwick*

[2] *Department of Chemistry, University of Warwick*

Corresponding author's email: Emma.Salisbury.1@warwick.ac.uk

Several approaches have been explored in an attempt to develop representative tissue models to study cell biology and diseases in vitro, including culture within a hydrogel (e.g. organoid culture) and culture on decellularised tissue. Nevertheless, these models do not fully recapitulate the complex tissue architectures, have limited physical and biochemical cues normally found in tissues and reproducibility is limited. Also, translatability of decellularised tissue-based scaffolds is limited due to intrinsic variability, the requirement for donor tissue and concerns over residual biological factors. Hence, the need for wholly synthetic scaffolds that incorporate sophisticated biochemical and mechanical cues as mimics of the native extracellular matrix and can ultimately provide robust platforms for the creation of 3D tissue models.This presentation will describe our recent developments of porous polymer scaffolds using emulsion-templating process. The droplets of a polymerisable high internal phase emulsion (HIPE) become interconnected upon chemical curing/crosslinking, producing a fully interconnected porous material, known as a polyHIPE. The pore diameter and properties of these materials can be readily tailored, making them suitable for 3D cell culture, tissue engineering and regenerative medicine. Non-degradable and fully degradable polyHIPE materials prepared by photochemical thiol-ene polymerisation will be presented[1]. The resulting scaffolds are similar chemically to common clinically used polyester biomaterials (e.g. polylactide and polycaprolactone). The mechanical properties of these materials can be tuned from highly elastic to highly rigid[2]. The scaffolds are capable of supporting 3D growth of many cell types including human pluripotent stem cells[3], human haematopoietic stem cells[4] and primary human endometrial cells[5].

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Abstract **#2142**

A SYSTEMATICALLY REDUCED MATHEMATICAL MODEL FOR ORGANOID EXPANSION

Meredith Ellis[P][1], Mohit Dalwadi[1], Marianne Ellis[2,3], Helen Byrne[1], Sarah Waters[1]

[1] *University of Oxford*

[2] *University of Bath*

[3] *Cellesce Ltd.*

Corresponding author's email: meredith.ellis@maths.ox.ac.uk

Organoids are three–dimensional multicellular tissue constructs that recapitulate the structure, heterogeneity, and function of their in vivo counterparts when cultured in vitro. As such, organoid production is a key technology in drug discovery and personalised medicine. A key challenge is producing organoids at scale in a reproducible manner. We are working with biotechnology company Cellesce, who develop bioprocessing systems for the expansion of organoids at scale. Part of their technology includes growing the organoids within a bioreactor, which utilises flow of culture media to enhance nutrient delivery to, and waste removal from, the organoids. Understanding how to optimise bioreactor operating conditions requires spatiotemporal information about the media flow and metabolite concentrations within the bioreactor. While it is impractical and expensive to collect this data empirically, mathematical modelling can be used to address these challenges. We present a model to investigate how mass transport of glucose and lactate within the bioreactor depends on the inlet flow rate and the cell seeding density [1]. We exploit the slender geometry of the domain to derive a long–wave approximation of the system. This reduced–order model systematically accounts for the depth–averaged flow, nutrient delivery, and waste removal, at a fraction of the computational cost of the full system. We explore the behaviour of the original and simplified models using analytical and numerical approaches and explain how our quantitative insights can be used to understand how organoid viability can be improved by varying specific bioreactor operating conditions.

Keywords

Organoid; Bioreactor; Multiscale

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Abstract **#2143**

DESIGN OF FREEZE-DRYING HYDROGEL DRESSING BASED ON NATURAL POLYSACCHARIDE GUM KARAYA FOR WOUND HEALING OF SKIN DEFECTS

Eva Černá[P][1], Lucy Vojtová[1]

[1] *CEITEC BUT*

Corresponding author's email: Eva.Cerna1@ceitec.vutbr.cz

Hydrogel based materials are promising candidates for effective treatment of skin defects (acute, chronic). Various materials, including synthetic and natural polymers, are widely used to produce gels, hydrogels, or thin films. This work is focused on the preparation of a cost-effective hydrogel dressing consisting of both natural and synthetic polymers. Natural polysaccharide Gum Karaya (GK) was chosen as a promising renewable resourced compound due to its low price, abundance, hydrophilicity, and potential antimicrobial properties. GK solution has been modified with hydrophilic poly(vinyl alcohol), and an emollient component to obtain a soft, transparent, and elastic dressing by freeze-drying.
The work aimed to design and production of a biocompatible hydrogel dressing suitable for long-term application to the wound site with pro-healing properties, nontoxicity, biomechanical properties, and transparency by mimicking the skin behaviour. Hydrogel dressings were characterized by a range of structural and mechanical properties including FTIR, UV/VIS, transparency, dynamic mechanical analysis, swelling behaviour, hydrolytic stability, biocompatibility, and morphology. Prepared dressings were non-toxic, transparent, flexible (elongation at break 150 % at the dry state and 380% at wet) and showed sufficient ability to swell (85% water content) with long-term hydrolytic stability (37 °C in physiological solution) for weeks, creating a suitable environment for wound healing management.

Keywords

Gum Karaya; wound dressing; skin-like

Abstract **#2144**

RHO/ROCK INHIBITION PROMOTES TGF-B3-INDUCED TENOGENIC DIFFERENTIATION IN MESENCHYMAL STROMAL CELLS

Michaela Melzer[P][1], Susanna Schubert[2], Simon Franz Müller[3], Joachim Geyer[3], Alina Hagen[1], Sabine Niebert[1], Janina Burk[1]

[1] *Equine Clinic (Surgery, Orthopedics), Justus-Liebig-University, 35392 Giessen, Germany*

[2] *Institute of Human Genetics, University of Leipzig Hospitals and Clinics, 04103 Leipzig, Germany*

[3] *Biomedical Research Center Seltersberg (BFS), Institute of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, 35392 Giessen, Germany*

Corresponding author's email: Michaela.Melzer@vetmed.uni-giessen.de

Mesenchymal stromal cells (MSC) represent a promising therapeutic tool for tendon regeneration. Their tenogenic differentiation is crucial for tissue engineering approaches and could be successfully induced by the transforming growth factor (TGF)-β, which is signalling through intracellular smad molecules. Moreover, scaffold topography or tendon matrix components mediated tenogenesis via activation of the rho/ROCK cascade, which, however, is also involved in pathological adaptations in extracellular matrix pathologies. This work investigated the interplay of rho/ROCK and TGF-β3/smad signalling in tenogenic differentiation in both human and equine MSC. Primary equine and human MSC were cultured as monolayers or on tendon-derived decellularized scaffolds to evaluate the influence of the ROCK inhibitor Y-27632 on TGF-β3-induced tenogenic differentiation. The MSC were incubated with and without TGF-β3 (10 ng/ml), Y-27632 (10 µM), or both. The signalling pathway of TGF-β and the actin cytoskeleton were visualized by smad 2/3 and phalloidin stainings, and gene expression of signalling molecules and tendon markers was assessed. ROCK inhibition was confirmed by disruption of the actin cytoskeleton. Activation of smad 2/3 with nuclear translocation was evident upon TGF-β3 stimulation. Interestingly, this effect was most pronounced with additional ROCK inhibition in both species ($p < 0.05$ in equine MSC). In line with that, the tendon marker scleraxis showed the strongest upregulation when TGF-β3 and ROCK inhibition were combined ($p < 0.05$ in human MSC). In conclusion, the results showed that ROCK inhibition promotes the TGF-β3/smad 2/3 axis, with possible implications for future MSC priming regimes in tendon therapy.

Keywords

transforming growth factor ; crosstalk; tendon-derived scaffold

Abstract **#2145**

BURN INJURY LEADS TO AN IMMEDIATE MULTI-LAYERED MOLECULAR RESPONSE, WITH GROWTH SIGNALING AN INTEGRAL COMPONENT WHICH DETERMINES TISSUE SURVIVAL

Nadja A R Ring[P][1], Helene Dworak[1], Barbara Schädl[2,3,4], Kornelia Schützenberger[2,3], Paul Slezak[2,3], Heinz Redl[1,2,3], Mikolaj Ogrodnik[1]

[1] *Ludwig Boltzmann Research Group SHoW – Senescence and Healing of Wounds, Vienna Austria*

[2] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre, Linz and Vienna, Austria*

[3] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

[4] *University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria*

Corresponding author's email: nadja.ring@trauma.lbg.ac.at

Burns are a prevalent healthcare problem, worsened by factors such as advanced age, diabetes or infections. Abnormal or delayed healing can lead to long-term sequelae and significant healthcare costs. It has been shown that skin burn damage can be ameliorated by proper diagnosis of burn severity and immediate treatment. Therefore, one of the key outstanding puzzles in the field is the molecular characterization of an early response to burns. Here, we propose a methodology for the prediction of cell fates in burned skin.

We use several models to study the early effects of burns on skin, including in vivo and ex vivo approaches on murine, porcine and human skin. Our in vivo porcine model allows us to study the immediate response to burns, with detailed histological assessment up to 6 h post-burn. Our complementary ex vivo technology opens the way for agonistic and inhibitory drug treatments to modulate the burn response. We observe a temperature-dependent multi-layered burn response, with zones of cell rupture, damage signaling and necrosis, apoptosis, DNA damage, and pro-growth signaling. We quantitatively describe a spatiotemporal response to heat injury including the depth of each molecular response and functional overlap between the various zones. The most striking change is a dramatic temperature-dependent activation of growth signaling pathways in the dermis and hypodermis. This activation represents the survival zone and is integral to the wound healing response. Our findings pave the way for the development of targeted treatments and diagnostic tools for more efficient management of burn injuries.

Keywords

burn; skin; growth signaling

Abstract **#2146**

ASSESSMENT OF THE BONE REGENERATION POTENTIAL OF SPIN-COATED ZINC SILICATE/POLYCAPROLACTONE COMPOSITES IN A WISTAR RAT FEMORAL OSTEONECROSIS BONE MODEL

Latifeh Karimzadeh[P][1,2], Jochen Salber[2,3], Ehsan Seyedjafari[4], Ghamartaj Hosein[1], Mohammad Nabiuni[5], Mohammadhosein Majlesara[6,7]

[1] *School of Biology, College of Science, University of Tehran, Tehran, Iran.*

[2] *Salber Laboratory, Centre for Clinical Research, Department of Experimental Surgery, Ruhr-Universität Bochum, Germany*

[3] *Department of Surgery, Universitätsklinikum Knappschaftskrankenhaus Bochum GmbH, Bochum, Germany*

[4] *University of Tehran, Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran.*

[5] *Department of Cell and Molecular Biology, Faculty of biological Sciences, Kharazmi University, Tehran, Iran.*

[6] *Faculty of Physics, Photonics Lab, Kharazmi University, Mofatteh Ave.,Tehran, Iran*

[7] *Applied Science Research Center(ASRC) Kharazmi University, Tehran, Iran*

Corresponding author's email: latifehkarimzadeh@gmail.com

Willemite (Zn_2SiO_4) is a bioceramic with bone defect healing properties. The aim of this study was to evaluate biocompatibility and osteogenic activity of nanoparticulate Willemite (npW) in Willemite/polycaprolactone (npW/PCL) composites. Porosity and crystallinity of npW/PCL were measured by TEM and XRD. The npW/PCL mixtures were spin-coated on 3D printed PCL scaffolds. Coating Thickness was determined by SEM. The topochemical and topomorphological changes of the scaffold surface were analysed using FTIR and XRD. The bioactivity of the npW/PCL composite was evaluated by immersion composite in simulated body fluid. In-vitro cytocompatibility studies were carried out by culturing rat bone marrow stem cells on the npW/PCL scaffolds for 72 hours. Composite scaffolds were fabricated with a geometry equal to the femur bone defects of Wistar rats and were placed in the target bone for two months and the newly formed bone was analyzed by µCT. the presence of npW in PCL increased the crystallinity and the hydrophilic functional groups compared to the neat PCL. The bioactivity studies showed that fewer but larger hydroxyapatite nuclei were formed on the npW/PCL composite than on the neat PCL. Scaffolds containing npW showed greater cytocompatibility after 72 hours than scaffolds without. Micro-CT analysis demonstrated that the npW/PCL scaffolds had a significantly higher bone ingrowth compared to neat PCL scaffolds at 8 weeks after implantation.This study was conducted to show the potential of Willemite nanoparticles in 3D printed porous npW/PCL composite scaffolds as a versatile approach to promote bone ingrowth and regeneration of osteonecrotic focus in rat femur.

Abstract **#2147**

SURFACE IMMOBILIZATION OF ANTIMICROBIAL CATH-2 PEPTIDE TO PREVENT IMPLANT-RELATED INFECTION

Pardis Keikhosravani[1], Fatemeh Jahanmard Hossein Abadi[1], Leonardo Cecotto[1], Saber Amin Yavari[1]
[1] *Department of Orthopedics, University Medical Center Utrecht, Utrecht, The Netherlands*
Corresponding author's email: p.keikhosravani@umcutrecht.nl

Introduction

Implant-associated infection (IAI) results from bacteria that initially colonize on the surface of the implants, then proceed to form a biofilm. Since bacteria growing in biofilms are tolerant to antibiotics and immune cell clearance, these infections are usually treated by removing the implant in a sophisticated two-steps revision surgery which imposes a huge socioeconomic burden [1]. Host defense peptides (HDPs) are considered as an interesting broad-spectrum alternatives to antibiotics in preventing IAI [2]. In particular, CATH-2 peptide (as one of the HDPs) causes a direct disruption of the microbial membrane thanks to its strong cationic charge [3]. Here, we aim to immobilize CATH-2 on porous titanium (Ti) surfaces using a carboxylic-based coating which can be applied via an electrospraying technique.

Materials and methods

A solution of propoxylated bisphenol A fumarate polyester (65 mg/ml) and Ethanol was coated by electrospray method to apply COOH group on Ti implants. CATH-2 (RFGRFLRKIRRFRPKVTITIQGSARF-NH2) was immobilized on the coated Ti through COOH bonding of polyester to amines (–NH2) of the peptide by 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) reaction.
In vitro antibacterial activity against Staphylococcus aureus, the peptide release behavior, and cytotoxicity of the coated implants were investigated by colony-forming unit (CFU) count, BCA protein kit, and Alamar blue assay, respectively.

Results and discussion

Our materials characterization and biological assays verified that the polyester coating has improved the peptide immobilization on the Ti surface, and significantly decreased bacterial adherence as well. This surface could be potentially applied on Ti implants in post-antibiotic era.

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Abstract **#2148**

FACILE APPROACH FOR IMAGING CELLS ON NON-TRANSPARENT POLYPYRROLE INCORPORATED SF/GEL MATRICES USING CLOVE DERIVED CARBON NANO DOTS

Gaurav Kulkarni[1,2], Preetam Guha Ray[2], Anurag Kumar Pandey[3,2], Santanu Dhara[2], Soumen Das[1]

[1] *Bio-MEMS Lab, School of Medical Science & Technology, IIT Kharagpur, Kharagpur-721302, West Bengal, India*

[2] *Biomaterials & Tissue Engineering Lab, School of Medical Science & Technology, IIT Kharagpur, Kharagpur-721302, West Bengal, India*

[3] *School of Nano-science & Technology, IIT Kharagpur, Kharagpur-721302, West Bengal, India*

Corresponding author's email: gaurav.kulkarni@iitkgp.ac.in

Fluorescence-based imaging of cell proliferation or adhesion on 3D/ porous scaffolds has limitations owing to its non-specific stain entrapment or its own autofluorescence behaviour. Herein, a facile route of imaging cell proliferation on non-transparent matrices was developed using polypyrrole (PPy) incorporated electroconductive gelatin-silk fibroin (Gel/SF) hydrogel. Interfacially polymerized PPy coated Gel/SF were further impregnated with carbon nano dots (CDs), synthesized using hydrothermal method from clove buds as a precursor. PPy was chosen due to its high conductivity or cytocompatibility while CDs are known for their tailorable fluorescence and excellent water solubility. Formation of CDs was first confirmed using UV-vis, which showed band corresponding to n–π* transition of multi-conjugate C=O of the CDs. Subsequently, L929 mouse fibroblast cells were seeded on as prepared formulation of PPy-Gel/SF. After 48 h, CDs were added at predefined concentration and samples were incubated for 8 h. Fluorescence imaging was performed to visualize cells on electroconductive matrix under UV (358 nm), green (540 nm), and red (650 nm) excitations. Moreover, single cell Raman signals were acquired so as to confirm intake of CDs by L929 cells. Two Raman lines associated with D-band and G-band were investigated for approving the presence of CDs. Thus, CD incorporated PPy based Gel/SF scaffolds can be deployed for facile and continuous monitoring of live or fixed cells on non-transparent/porous scaffolds.

Abstract **#2149**

SYNTHETIC PEPTIDE HYDROGELS AS EXTRACELLULAR SUPPORTS FOR THE DERIVATION AND SPECIFICATION OF HIPSC-DERIVED KIDNEY ORGANOIDS

Shane Clerkin[P][1,2], Niall Treacy[1,2], Jessica Davis[1,2], Ciaran Kennedy[1,2], Jacek Wychowaniec[3], Dermot Brougham[3], John Crean[1,2]

[1] *Diabetes Complications Research Centre, Conway Institute of Biomolecular and Biomedical Science*

[2] *University College Dublin (UCD) School of Biomolecular and Biomedical Science, Belfield, Dublin 4, Ireland*

[3] *UCD School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland*

Corresponding author's email: shane.clerkin@ucdconnect.ie

The directed differentiation of induced pluripotent stem cells (iPSCs) to kidney organoids has facilitated pioneering studies into processes that underpin both human kidney development and the pathogenesis of disease. Continued advances in stem cell technologies have made the provision of patient-specific, bioengineered replacement organs a realistic prospective treatment for end-stage renal disease. Synthetic biomaterials represent a promising means of generating physiologically relevant, non-xenogeneic and mechanically tuneable microenvironments conducive towards improved complexity and maturity of tissue constructs. Within this context, we differentiated iPSC-derived nephron progenitors into kidney organoids within synthetic self-assembling peptide hydrogels of variable mechanical strengths. Histological analysis of kidney organoids revealed variable self-organisation of tubular epithelia and interstitial cells by day 24 of differentiation, most notably in peptide hydrogels with Storage Moduli (G') of 1 and 14 kPa. Immunofluorescent characterisation of the organoids confirmed the formation of glomerular, proximal tubular and distal tubular structures, supported by basement membrane and interstitial cells. Single-cell RNA sequencing was subsequently used to delineate the effects of the biophysical environment on the differentiation and fate of cells. Following unsupervised clustering, 13 distinct cell types were identified. Most notably, stromal expansion was shown to vary significantly between organoids differentiated within soft vs stiffer synthetic microenvironments. To the best of our knowledge, this is the first investigation of iPSC-derived kidney organoids within fully synthetic, self-assembling peptide hydrogels. These results will further support the generation of designer matrices that will improve maturity and the translational potential of kidney organoids.

Keywords

Kidney; Organoid; Peptide Hydrogel

Abstract **#2150**

A SIMPLE AND EFFICIENT ALTERNATIVE TO THE TRADITIONAL COLLAGEN GEL MODEL TO STUDY THE TRANSITION FROM OSTEOBLASTS TO OSTEOCYTES

Pragnere SarahP[1], Emma Petiot[2], Cyril Pailler-Mattei[1,3]

[1] *University of Lyon, Laboratory of Tribology and Dynamic of Systems, UMR-CNRS 5513, ENISE - ECL, 36 Avenue Guy de Collongue, Ecully, 69134, France*

[2] *3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, Bat. Lederer, 1 rue Victor Grignard, Villeurbanne, 69100, France*

[3] *University of Lyon, University of Claude Bernard Lyon 1, ISPB-Faculté de Pharmacie, Lyon, F-69008, France*

Corresponding author's email: sarah.pragnere@ec-lyon.fr

Osteocytes require 3D environment to maintain their phenotype in vitro (1). Due to the difficulty to isolate and grow these cells, most studies have so far have used cell lines (2–4). Alternatively, studies have focused on the use of collagen gel to embed the cells and differentiate primary osteoblasts (1,2,5). We demonstrate the use of a new hydrogel that supports primary osteoblasts differentiation to osteocytes. Primary human osteoblasts were embedded at a density of 300 000 cells/mL in a bioink composed of fibrinogen, gelatin and alginate polymerized by calcium and enzymatic solution. Cells remained viable during the 50 days of the experiment even in the center of the tissue and displayed characteristic features of osteocytes: no cell growth, dendrites formation and expression of PHEX, a histological marker of osteocyte (6). Unlike the collagen model which requires a high density (1.106 cells/mL) of osteoblasts to induce differentiation from osteoblasts to osteocytes (7), we managed to differentiate the cells with a lower density. As primary cells are difficult to obtain and have a very limited proliferation capacity, this is of major convenience. Moreover, this biomaterial is printable and provides high shape fidelity. Therefore it could be used to reproduce the porous trabecular bone. The polymerization step can also be adjusted to tune the mechanical properties of the hydrogel (elastic modulus from 1kPa to 80kPa), allowing to examine the response of the cells to their environment stiffness.

Keywords

osteoblasts; osteocytes; differentiation

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Abstract **#2151**

NEW CRYOPRESERVATION METHOD FOR 3D HEPATOCYTE CULTURE MODELS

Alba Herrero-Gómez$_P$[1], Marc Azagra[1], Alba Riba[1], Jose Yeste[1], Irene Marco-Rius[1]

[1] *Institute for Bioengineering of Catalonia*

Corresponding author's email: aherrero@ibecbarcelona.eu

Cryopreservation methods for cell and tissue storage have been around since 1954, where thawed sperm samples were used for an insemination. Since then, the technology has evolved for clinicians and researchers to cryopreserve tissue, cell lines and primary cells. While cryopreserving tissues helps maintain their physiological integrity for study it does not assure the viability of the cells after thawing. Furthermore, the cells cryopreserved in suspension lose their dimensional anchors, forcing them to change their morphology. To solve this issue, tissue engineering allows researchers to create 3D culture models, such as organoids and bioprinted cell clusters, that mimic the physiological characteristics of the cells in tissue and disease. Although this culture methods present promising results, there is a lack of methodology to cryopreserve 3D cell models and patients' samples for storage and transport in a way where they remain viable after thawing. We propose a protocol that uses a carboxymethyl cellulose scaffold and precise freezing and thawing conditions for spheroid survival. The scaffold provides structure for the hepatocytes to create spheroids on their own as well as support throughout the freezing and thawing processes for optimal cell viability post-thawing. Furthermore, this method will achieve higher cell viability than transporting the cells as a cryopreserved pellet for model assembling after thawing, allowing the cells to settle and form a tissue beforehand to improve viability after cryopreservation. This technique constitutes a step forward for it will facilitate the transport of already assembled 3D models from cell lines or primary cells from patients.

Keywords

Cryopreservation

Abstract **#2152**

EVALUATION OF DIFFERENT TREATMENT STRATEGIES FOR ACUTE LIVER FAILURE BASED ON A PERFUSED BED BIOREACTOR

Claire de Lartigue$_P$[1], Ulysse Pereira[1], Pasqua Mattia[1], Jonathan Nicolas[1], Joëlle Rialland[1], Hippolyte Boucher[1], Manon Guillet[1,2], Cécile Legallais[1,3]

[1] *UMR CNRS 7338 Biomechanics & Bioengineering, Université de Technologie de Compiègne, 60203 Compiègne, France*

[2] *UR 2223 Connaissance Organisation et Systèmes TECHniques, Université de Technologie de Compiègne, 60203 Compiègne, France*

[3] *FHU Hepatinov, Villejuif, France*

Corresponding author's email: claire.de-lartigue@utc.fr

Bioartificial liver remains a promising alternative to liver transplantation for acute liver failure (ALF). We have recently demonstrated that the HepaRG cells encapsulated in alginate beads hosted in a perfused dynamic bioreactor (PDB) meets the clinical needs in terms of liver functions (1,2). In this study, we aim at evaluating the PDB under near-real conditions by mimicking moderate and severe ALF with several therapy modalities.

HepaRG cells were encapsulated in alginate beads by coaxial airflow extrusion and then transferred to PDB for orbital shaking in classical culture medium. Starting from day 14, cells' performances were assessed during three successive therapies of 6 hours (one per day) or a 72h therapy in moderate and severe ALF in vitro models. Different parameters were measured such as viability, albumin synthesis and ammonia detoxification rate.

Encapsulated cells remained viable and demonstrated their capacity to maintain activities after three days of therapy. Albumin production remained stable (around 2.6 µg/6h/million cells) while ammonia detoxification was higher in a severe pathologic model than in a moderate one (respectively 213 and 112 mmol/6h/million cells).

Interestingly, when we get closer to the clinical therapy conditions, cells become more efficient. It is also very important to note that cells can be used for three successive therapies. These results will help us to launch animal experiments in accordance with clinicians needs and regulatory agencies.

The research project is funded by ANR 16-RHUS-0005 in the iLite programme. The HepaRG cell line were provided by Biopredic International.

Keywords

Liver supply; Bioreactor; Alginate

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Abstract **#2153**

CHARACTERIZATION OF NATIVE TENDON SCAFFOLDS AS IN VITRO MODEL FOR CHRONIC TENDON DISEASE

Carla Doll$_P$[1], Kerstin von Pueckler[2], Julia Offhaus[3], Janina Burk[1]

[1] *Equine Clinic (Surgery, Orthopedics), Faculty of Veterinary Medicine, Justus-Liebig-Universitiy, Giessen, Germany*

[2] *Small Animal Clinic (Surgery, Radiology), Faculty of Veterinary Medicine, Justus-Liebig-University, Giessen, Germany*

[3] *Department for Horses, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany*

Corresponding author's email: carla.u.doll@vetmed.uni-giessen.de

In acute tendon lesions, the effect of mesenchymal stromal cells (MSC) is mainly attributed to immunomodulatory mechanisms. However, little is known about their mode of action in chronic disease. Therefore, we aimed to establish a cell culture model that reflects extracellular matrix alterations in chronic tendon disease. Equine limbs were obtained from an abattoir and superficial digital flexor tendons were dissected. Based on macroscopic and ultrasonographic evaluation 17 tendons were chosen to be examined in a 0.27T standing equine-dedicated low-field magnetic resonance imaging (MRI) system. Subsequently, 12 of these tendons were also examined in a 3T MRI in horizontal position as usual for the 3T MRI, and in standing position corresponding to the procedure in the equine system. Tendons were decellularized to be used as scaffolds for MSC culture. Tendon disease was detected with both MRI systems, with differences in lesion discrimination and signal intensities between horizontal and standing positions. N=6 tendons with increased signal in T1 sequences and normal signal in T2 sequences were chosen to model chronic tendon disease in vitro. N=6 healthy tendons served as controls. Cultivation of MSC on tendon scaffolds altered their extracellular matrix gene expression. Based on preliminary data, we also expect adaptations to the diseased matrix environment.

Keywords

chronic tendon disease; equine

Abstract **#2154**

WHAT IS A BIOLOGICALLY INSPIRED HUMAN-DESIGNED LIVER?

Manon Guillet[P][1,2], Ulysse Pereira[1], Claire de Lartigue[1], Cécile Legallais[1,3], Xavier Guchet[2]

[1] *UMR CNRS 7338 Biomechanics & Bioengineering, Université de Technologie de Compiègne, 60203 Compiègne, France*

[2] *UR 2223 Connaissance Organisation et Systèmes TECHniques, Université de Technologie de Compiègne, 60203 Compiègne, France*

[3] *FHU Hepatinov, Villejuif, France*

Corresponding author's email: manon.guillet@utc.fr

To overcome global graft shortage, researchers try tackling the issue by developing human-designed organs (as opposed to native ones): devices/methods aiming to temporally assist or permanently replace the liver. To understand how they were conceived, we undertook a philosophical analysis of the field. According to findings, designers start from what they know. The first source of inspiration is nature, in this case the native liver, or, more exactly, the conception that they have of it: is the organ defined by its structure (lobule, sinusoid…)? Its functions (detoxification, metabolization…)? Both or neither? The second source of inspiration is pre-existing technologies/methods, such as dialysis or cellular culture. From those two inspirations, researchers develop design strategies: for example, conceiving purely artificial devices in order to avoid all the constraints posed by cells, or on the contrary, using cells by betting that, even if we do not completely understand their functioning, they will act as they do in vivo. Questioning current scientific methodologies could allow burgeoning of further inclusive design approaches by incorporating other inspirations and strategies. Integrating this momentum, is accepting that technique is not neutral, that technical objects carry the conceptions and values of those who created them(1) and, thus, they shape humans and the world.

Keywords

Liver Supply; Design; Philosophy of science

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Abstract **#2155**

MULTIFUNCTIONAL COLLAGEN MIMETIC MATERIALS FOR BOTTOM-UP CONTROL OF CELLULAR MICROENVIRONMENTS

April M. Kloxin[P][1]

[1] *University of Delaware*

Corresponding author's email: akloxin@udel.edu

Characteristics of tissues throughout the body vary based on tissue type and function, for example, from the collagen-rich compliant, loose connective tissues of the lung to the stiff, regular connective tissues of the tendon and ligament. The physical properties of these tissues arise in part from the assembly and hierarchical organization of large structural proteins and glycosaminoglycans. In particular, collagen-rich tissues rely heavily on the self-assembly of smaller collagen subunits to form multiscale structures of fibrils and fibers that are further organized and modified by cells. This bottom-up (e.g., assembly) and top-down (e.g., cellular remodeling) organization of the extracellular matrix allows adaptation of tissue properties in response to stimuli in reversible (e.g., strain hardening) or directional (e.g., fibrotic stiffening) manners. These complex systems provide inspiration for the design of engineered materials with hierarchical structures and tunable and adaptable mechanical properties, presenting opportunities for creating improved tissues mimics of relevance for studying and directing cellular responses in vitro and in vivo. Specifically, in this talk, I report on our recent efforts in the design and application of multifunctional collagen mimetic peptide (mfCMP) based materials. These mfCMP materials systems integrate collagen mimetic peptides with different reactive handles for crosslinking, modification, and dynamic modulation of the properties of hydrogel-based synthetic extracellular matrices in response to light and temperature. Further, the biophysical and biochemical properties of these materials can be tailored to promote cell spreading and motility and to stabilize injured collagen-rich tissues.

Keywords

collagen mimetic peptides; hydrogel; connective tissues

Abstract **#2157**

A FUNCTIONAL 3D SKELETAL MUSCLE MODEL FOR DUCHENNE MUSCULAR DYSTROPHY FOR THE EVALUATION OF POTENTIAL THERAPIES

Ainoa Tejedera-Villafranca[1], Lluís Mangas-Florencio[1], Jose Yeste[1], Javier Ramón-Azcón[1,2], Juan M. Fernández-Costa[1]

[1] *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), C/Baldiri Reixac, 10-12, E-08028 Barcelona, Spain*

[2] *ICREA-Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain*

Corresponding author's email: atejedera@ibecbarcelona.eu

Research into the development of therapeutic strategies is based mainly on animal models and cell cultures. The ability to extrapolate data from them is limited, and research on new drugs cannot be performed efficiently. This is especially dramatic in rare diseases, which are intrinsically very heterogeneous. The generation of advanced models using tissue engineering and patient-derived cells allows fabricating new platforms for studying pathological processes and discovering new potential drugs. Here, we developed a patient-derived 3D functional skeletal muscle for Duchenne muscular dystrophy (DMD). DMD is the most prevalent neuromuscular disease diagnosed during childhood. The disease is characterized by progressive degeneration of skeletal and cardiac muscle caused by the lack of dystrophin protein. Although there are several molecules in drug development for DMD, there is no treatment available for patients to date. By using a 3D-printed casting mold, we encapsulated patient-derived myogenic precursor cells in a fibrin-composite matrix. This platform incorporated two flexible T-shaped pillars that provided continuous tension to the tissue, thus allowing the orientation of the muscle fibers. Our 3D muscle model expressed mature muscle markers and responded to electric pulse stimulation (EPS). Besides, contraction dynamics between DMD and control tissues were shown to be different. Moreover, an increase of damage markers after EPS was observed in DMD but not in healthy tissues. Finally, the tissues will be integrated into a microfluidic device to monitor drug administration. Eventually, the microfluidic system will be connected to a biosensors system for the real-time detection of biomarkers.

Keywords

Casting; Muscular dystrophy; Contraction dynamics

Abstract **#2158**

MULTIFUNCTIONAL AND BIOACTIVE ALGINATE-BASED MATRIX FOR BRAIN TISSUE BIOENGINEERING

Natalia Gostynska$_P$[1], Giulia Della Rosa[1], John Wesley Ephraim[1], Monica Moroni[2], Gabriella Panuccio[1], Nicola Tirelli[3], Gemma Palazzolo[1]

[1] *Enhanced Regenerative Medicine, Istituto Italiano di Tecnologia, Genova, Italy*

[2] *Neural Computation Laboratory, Istituto Italiano di Tecnologia, Rovereto, Italy*

[3] *Laboratory of Polymers and Biomaterials, Istituto Italiano di Tecnologia, Genova, Italy*

Corresponding author's email: natalia.gostynska@iit.it

The ideal biomaterial for brain tissue engineering (TE) should provide versatile features which together play a synergistic role in the regeneration of the damaged brain matter. A successful candidate should not only mimic the architecture and composition of the native tissue but also protect the surroundings from excitotoxicity and oxidative stress, which are critical factors in brain injuries. Here, we address these features by exploiting the multi-active role of magnesium ions to improve both physico-chemical and biological properties of alginate biopolymer and finally develop an alginate-based biosystem for brain TE. For the first time, the calcium-driven inner gelation of magnesium alginate (MA) provided soft and homogeneous alginate-based hydrogels. 3D cultures of rat embryonic primary neurons demonstrated that MA is cytocompatible, supporting neuronal network formation. Already after 7 days in vitro (DIV), cells encapsulated in the MA hydrogels showed a higher number of total neurite length and branch points compared to commonly used sodium alginate (SA). Moreover, neurons in MA manifested a higher level of functionality after 28 DIV in terms of spontaneous neuronal activity evaluated by calcium imaging. Further, we explored the anti-oxidant and neuroprotective role of the MA in a 2D system using rat embryonic neural stem cells. These experiments showed that MA protects the cells against oxidative stress reducing cell mortality, biomarker degradation and reactive oxygen species production. Taken together, these results indicate that the MA ion-responsive polymer exhibits multiple bioactive roles in both 2D and 3D cellular systems, supporting its use for brain tissue bioengineering.

Keywords

brain tissue engineering ; neuronal networks; neuroprotection

Abstract **#2159**

BIO-INK FORMULATION AND PRINTING FIDELITY OF FMOC-DIPHENYLALANINE (FMOC-FF) DIPEPTIDE HYDROGELS FOR BIOPRINTING BONE TISSUE GRAFTS

Ayse Kose[P][1,2,3], Paulo Amorim[2,4], Olivier Deschaume[5], Alexander Volodin[6], Carmen Bartic[7], Liesbet Geris[1,2,3,8]

[1] *Prometheus Division of Skeletal Tissue Engineering, Department of Materials Science, KU Leuven, Belgium*

[2] *Skeletal Biology and Engineering Research Center, KU Leuven, Belgium*

[3] *Biomechanics Section, KU Leuven, Belgium*

[4] *Materials Technology TC, Campus Group T, KU Leuven, Andreas Vesaliusstraat 13, Leuven, Belgium*

[5] *Soft-Matter Physics and Biophysics Section, KU Leuven, University of Leuven, Leuven,*

[6] *Department of Physics and Astronomy, KU Leuven, Celestijnenlaan 200 D, B-3001, Leuven, Belgium*

[7] *Soft-Matter Physics and Biophysics Section, KU Leuven, University of Leuven, Leuven, Belgium.*

[8] *Biomechanics Research Unit, Université de Liège, Belgium*

Corresponding author's email: ayse.kose@kuleuven.be

Due to rapid developments in Additive Manufacturing (AM), 3D (bio)printing (especially extrusion bio-printing) is increasingly used for tissue engineering and regenerative medicine. Extrusion bio-printing requires the shear thinning hydrogels to reliably print certain geometry. Hydrogels are versatile molecules for 3D tissue engineering, however, when they are utilized as bio-inks, the rheological and mechanical properties are challenging to print with high shape and geometry fidelity and usually require additional crosslinking.

Peptide self-assembly is a novel way of forming stable hydrogels for scaffold manufacturing, 3D bio-printing and drug delivery applications. Among these peptides, Fmoc-diphenylalanine (Fmoc-FF) is highly investigated as a scaffold material due to its biocompatibility and interesting mechanical properties, which have been shown to positively influence osteogenesis and chondrogenesis. However, although Fmoc-FF is a very promising peptide, printability and bio-ink characteristics have not been well elucidated.

In this study, we use Fmoc-FF as a bio-ink material to investigate its' printability in an extrusion printing set-up as well as the use of these peptide hydrogels in bone tissue engineering. The peptide was blended with alginate in various concentrations to create a printable material allowing for high cell viability. Alginate/Fmoc-FF bio-inks were successfully printed into various geometries. Printability of the bio-inks was calculated with respect to feed and flow rate. Surface characterization was performed using atomic force microscopy (AFM), scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). Mechanical characteristics and rheological properties of the bio-inks are being evaluated. Taken together, we conclude that Alginate/Fmoc-FF hydrogels are versatile bio-inks for 3D bio-printing application.

Abstract **#2160**

ELECTROSPINNING OF FMOC-FF PEPTIDES TO CREATE PIEZOELECTRIC NANOFIBER SHEETS FOR BONE TISSUE ENGINEERING APPLICATIONS

Ayse Kose[P][1,2,3], Mahtab Asadian[2,4], Olivier Deschaume[5], Alexander Volodin[6], Carmen Bartic[7], Liesbet Geris[1,2,3,8]

[1] *Prometheus Division of Skeletal Tissue Engineering, Department of Materials Science, KU Leuven, Belgium*

[2] *Skeletal Biology and Engineering Research Center, KU Leuven, Belgium*

[3] *Biomechanics Section, KU Leuven, Belgium*

[4] *Research Unit Plasma Technology (RUPT), Department of Applied Physics, Ghent University, Belgium*

[5] *Soft-Matter Physics and Biophysics Section, KU Leuven, University of Leuven, Leuven, Belgium*

[6] *Department of Physics and Astronomy, KU Leuven, Celestijnenlaan 200 D, B-3001, Leuven, Belgium*

[7] *Soft-Matter Physics and Biophysics Section, KU Leuven, University of Leuven, Leuven, Belgium.*

[8] *Biomechanics Research Unit, Université de Liège, Belgium*

Corresponding author's email: ayse.kose@kuleuven.be

Diphenylalanine (FF) has been shown to function as a piezoelectric material with significant mechanical stiffness. The piezoelectricity of the diphenylalanine is arising from the π-π stacking in between the aromatic residues. Currently, due to high piezoelectricity, Fmoc-FF (chemical synthesis with Fmoc protecting group) is utilized for creating micro-nanogenerators and bio-organic conduits for energy harvesting purposes.

Bone is a piezoelectric tissue due to the collagen assemblies in extracellular matrix (ECM) environment. According to Wolff's law, bone adapts its architecture to the mechanical loading it is subjected to. Although the role of the mechanical loading is not fully explained, it is known that piezoregulation is one of the factors active in bone homeostasis. Piezoelectric materials create an environment for mechanical-electrical coupling, which contributes to the bone remodeling process. Fmoc-FF as material for 3D tissue engineering, has been shown to aid osteoblastic differentiation. However, the effect of its piezoelectricity on osteogenesis has never been addressed.

To understand the role of piezoelectric effects of Fmoc-FF on osteogenesis, a nanofibrous Fmoc-FF network has been created using electrospinning. Fmoc-FF has successfully electrospun into nanofibers (80-150 nm). The morphology, mechanical properties, piezoelectricity and chemical characteristics of the Fmoc-FF sheets were assessed by scanning electron microscopy (SEM), , piezoresponse force microscopy (PFM), water contact angle measurements and Fourier-transform infrared spectroscopy (FTIR), respectively. Biological tests are ongoing. In conclusion, Fmoc-FF electrospun in sheets is a potentially interesting material to design novel scaffolds with proper mechanical and electrical properties for bone tissue engineering.

Abstract **#2161**

ADVANCED IN VITRO MANAGEMENT OF 3D CELL CULTURES AND TISSUES

Sebastian Kress$_P$[1], Dominik Egger[1], Cornelia Kasper[1]

[1] *University for Natural Resources and Life Sciences, Vienna*

Corresponding author's email: Sebastian.kress@boku.ac.at

3D In vitro tissue and organ models gain increasing importance due to the 3R rules and to deliver more relevant results mimicing the human cellular pathways and interactions for a clinical translation.
However, there is currently no bioreactor or monitoring system commercially available, which facilitates a non-destructive long-term repetitive insight within the core of a 3D model.
The combination of open flow microperfusion (OFM) with a custom designed perfusion bioreactor and implemented sensors, enables a direct time resolved insight within the metabolic processes of a 3D tissue model. Furthermore, the setup serves as a minimally invasive sampling technology to yield highly concentrated metabolome and secretome equivalent to the interstitial fluid within the tissue. This facilitates the verification of metabolic and secretory processes, which are diluted below the detection limit when relying on analyzing the media supernatant. Moreover, the system is designed as a platform technology and by 3D printing customizable to various tissue geometries, sizes, and requirements allowing the models to be cultured mimicking physiologic conditions.

Abstract **#2162**

PROCESS PARAMETERS FOR CELL FREE THERAPY USING AUTOLOGOUS EVS

Sebastian Kress$_P$[1], Ciarra Almeria[1]

[1] *University of Natural Resources and Life Sciences, Vienna*

Corresponding author's email: Sebastian.kress@boku.ac.at

The use of extracellular vesicles (EVs) for cell free therapy present various advantages over cell based therapies. Many regenerative mechanisms are transmitted via secreted signals than direct cell-cell contact making the use of cell transplantion obsolete and thereby avoiding the respective risks. Nevertheless, the cellular production of EVs depends on the environmental conditions of the cells. Hence, the production of biologically active and therapeutically potent EVs requires culture conditions for the producing cells to induce and stimulate the desired functionality.
In a first study we evaluated various culture conditions for primary isolated MSCs upon their impact on EVs production, size distribution, cargo, and biological functionality.

Abstract **#2163**

BIOFABRICATION OF ANISOTROPIC CARTILAGE TISSUE USING STANDING BULK ACOUSTIC WAVES WITHIN HYBRID GELMA MATRICES

Parth Chansoria[1,2,3], Suleman Asif[1], Rohan Shirwaiker[1]

[1] *North Carolina State University, Raleigh, NC, USA*

[2] *University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

[3] *Eidgenössische Technische Hochschule Zürich, Zürich, CH*

Corresponding author's email: pchanso@ncsu.edu

In tissue engineering, it is important to recapitulate the microstructural organization of native tissues that is critical to their function[1]. Scaffold-based techniques have focused on achieving this via contact guidance principle wherein topographical cues offered by scaffold fibers direct migration and orientation of cells to govern subsequent cell-secreted extracellular matrix organization[2]. In contrast, this work describes a new ultrasound-assisted biofabrication (UAB) process that utilizes radiation forces generated by superimposing ultrasonic bulk acoustic waves to preferentially organize cellular arrays within single and multi-layered hydrogel constructs[3,4]. Specifically, we investigated the UAB of anisotropic cartilage constructs with patterned human adipose-derived stem cells or chondrocytes encapsulated within GelMA matrices. The cells organized in parallel arrays remained 100% viable post-UAB ($p < 0.0001$), and the metabolic activity was not affected compared to the groups without ultrasound exposure. The inter-cellular array spacing was also closely correlated with the theoretical estimate of half the ultrasound wavelength ($p < 0.001$). In addition to cells, we patterned collagen microaggregates and polycaprolactone microfibers within the GelMA matrix, which enhanced the mechanical stiffness, stretchability, and strength ($p < 0.05$) of the hydrogels while providing additional adhesion cues for cell proliferation and aligned ECM production. The results of long-term maturation of cell-laden constructs under the relevant chondrogenic culture medium clearly demonstrated that the hybrid constructs were superior to pure GelMA counterparts in maintaining construct stability and promoting cell alignment and anisotropic collagen-II deposition, and biomimicking the native organization of cells and collagen within cartilaginous tissues such as the annulus fibrosus and meniscus.

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Abstract **#2164**

MAGNESIUM-TAILORED ALGINATE AS A NOVEL BIOPOLYMER FOR BRAIN REPAIR

Gemma Palazzolo$_P$[1], Giulia Della Rosa[1], Natalia Gostynska[1], Stefania Bartoletti[2], Elisa Ren[2], Giuseppina Leo[2], Ilaria Decimo[3], Nicola Tirelli[4], Giulia Curia[2]

[1] *Enhanced Regenerative Medicine, Istituto Italiano di Tecnologia, 16163 Genoa, Italy*

[2] *Laboratory of Experimental Epileptology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy*

[3] *Section of Pharmacology, Department of Diagnostics and Public Health, University of Verona, 37134 Verona, Italy*

[4] *Polymers and Biomaterials, Istituto Italiano di Tecnologia, 16163 Genoa, Italy*

Corresponding author's email: gemma.palazzolo@iit.it

The algal polysaccharide sodium alginate represents a highly biocompatible and low immunogenic biomaterial offering a broad range of biomedical applications[1]. In brain tissue engineering, it has been employed to generate very soft hydrogels promoting neural stem cell differentiation and maturation of functional neuronal networks[2-5]. However, sodium alginate gelation, mediated by bivalent cations like calcium, is rather rapid and it leads to uneven and unstable hydrogels limiting their in vivo injectability. To overcome these issues, we carried out an ion substitution process to exchange monovalent sodium ions with bivalent non-gelling magnesium ions and we characterized, via rotational rheology and microrheology, the polymer physico-mechanical properties. Magnesium ions reduced the intrachain repulsion and increased the intramolecular interactions, in turn influencing alginate conformation and behaviour. Magnesium alginate was less viscous than sodium alginate and, in the presence of calcium, it led to slower gelation kinetics providing more homogenous and stable hydrogels. The improved resistance to deformation made magnesium alginate especially suitable for in vivo injection in the rat brain, through small infusion needles (26-28 gauge) allowing less invasive neurosurgery. In addition, being magnesium neuroprotective[6], we sought whether it could augment alginate anti-inflammatory action in brain tissue. To this aim, we performed immunohistochemistry of reactive gliosis biomarkers, upon either sodium or magnesium alginate treatment, in pathological conditions such as temporal lobe epilepsy and cytotoxic brain lesion induced in rats. Overall, this study demonstrated that magnesium alginate can provide both structural and trophic support to neural cells and revealed its potential for brain tissue repair.

Keywords

alginate ; rheology; anti-inflammatory

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Abstract **#2165**

ARTEMISININ LOADING IMPROVES THE HEMOCOMPATIBILITY AND CELL VIABILITY OF CERIUM-DOPED MESOPOROUS SILICA WITH HUMAN PERIODONTAL LIGAMENT FIBROBALSTS

IOANNIS TSAMESIDIS[P][1], DIMITRIOS GKILIOPOULOS[2], ANNA THEOCHARIDOU[1], ELEANA KONTONASAKI[1]

[1] *Aristotle University of Thessaloniki, Department of Dentistry, Thessaloniki, Greece*

[2] *Aristotle University of Thessaloniki, Department of Chemistry, Thessaloniki, Greece*

Corresponding author's email: johntsame@gmail.com

Artemisinin (ART) and its derivatives, artemisinins (ARTs), are the most useful antimalarial drugs presenting no side effects1. They probably activate the endoperoxide bridge by producing radicals via mechanisms associated with iron-activation, having half-live ranging between 2 to 5 hours2. The encapsulation of ARTs in mesoporous silica (MSNs) for sustained release represents a non-investigated way to improve the pharmaceutical properties of ARTs. Mesoporous calcium-silicate MSNs doped with cerium (1, 2.5, 5%) were synthesized via sol-gel (Ce-MSNs). ART-loaded (12.5–500μg/ml) Ce-MSNs were investigated in terms of biocompatibility with human periodontal ligament cells (hPDLs) and hemocompatibility with red blood cells. ART-loaded Ce-MSNs didn't present hemolytic activity at concentrations up to 1 mg/ml, while ART concentrations ranging from 100 nM to 1 mM were hemocompatible too. The MTT assay revealed that ART-loaded Ce-MSNs enhanced hPDLCs' proliferation, while 1 and 2.5% Ce-MSNs significantly promoted mitochondrial activity. ART is a promising candidate for the treatment of patients with dental-related microorganisms causing pulpitis, periodontitis, and peri-implantitis3. In the light of previous results for the role of artemisinin in osteogenesis3 and the enhancement of osteogenic differentiation in human mesenchymal stem cells4, our study provides the first link between loading of ART in MSNs and hPDLCs proliferation and sheds novel light on developing ART-cerium doped MSNs as promising candidates for bone regeneration in patients affected by peri-implantitis.

Acknowledgments

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Keywords

ARTEMISININ DRUG LOADING; CERIUM-DOPED MESOPOROUS SILICA; HUMAN PERIODONTAL LIGAMENT FIBROBALSTS

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Abstract **#2166**

EVALUATION OF EQUINE PLATELET LYSATE FOR MULTIPOTENT MESENCHYMAL STROMAL CELL CULTURE

Alina Hagen[P][1], Hendrik Lehmann[2], Vivian-Pascal Brandt[3], Heidrun Holland[3], Michaela Melzer[1], Julia Moellerberndt[1], Christiane Liliane Schnabel[4], Janina Burk[1]

[1] *Equine Clinic (Surgery, Orthopedics), Justus-Liebig-University Giessen, Giessen, Germany*

[2] *Department of Veterinary Clinical Sciences, Small Animal Clinic, Justus-Liebig-University Giessen, Giessen, Germany*

[3] *Saxonian Incubator for Clinical Translation (SIKT), University of Leipzig, Leipzig, Germany*

[4] *Faculty of Veterinary Medicine, Institute of Immunology, Leipzig University, Leipzig, Germany*

Corresponding author's email: Alina.Hagen@vetmed.uni-giessen.de

Fetal bovine serum (FBS), which is the current gold standard as a basal medium supplement for the cultivation of many cell types, including equine MSC, is afflicted with several problems. Different alternatives are being tested, with promising results using platelet lysate in human MSC culture. The aim of this study was to establish a standardized protocol for equine platelet lysate (ePL) production and to test the ePL in equine MSC culture. Whole blood collected from 20 healthy horses was processed into platelet concentrates using a buffy coat method. In these concentrates, the cells were lysed by freeze/thaw cycles. The pooled ePL from all donors was evaluated as culture medium supplement in comparison with FBS, using equine adipose-derived MSC. Platelet, platelet-derived growth factor BB and transforming growth factor β1 concentrations were increased in the processed concentrates compared with whole blood or serum ($p < 0.05$), while white blood cells were reduced ($p < 0.05$). When the ePL obtained was used at the same concentration (10%) as FBS, MSC expansion and adipogenic and osteogenic differentiation were supported similarly. In passage 5 MSC, karyotype analyses by GTG-banding suggested a higher genetic stability in the MSC cultured with ePL.

Keywords

mesenchymal stromal cells; platelet lysate; equine

Abstract **#2167**

TUNING CELLULAR MORPHOLOGY BY CHANGING STIFFNESS, PLASTICITY, DEGRADABILITY, AND BIOADHESION OF HYALURONIC ACID-BASED HYDROGELS

Fatemeh Rasti Boroojeni[1], Robert Selegård[1], Daniel Aili[1]

[1] *Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, Sweden*

Corresponding author's email: Fatemeh.rasti.boroojeni@liu.se

Hydrogels have been widely used to engineer the microenvironment of cells in the field of tissue engineering and regenerative medicine. The properties of the microenvironment, such as stiffness, plasticity, degradability, and bioadhesion, will among others affect cell morphology, migration, and proliferation. However, controlling cell morphology and behavior in a defined manner by an engineered microenvironment is still challenging. Using a matrix metalloprotease (MMP)-sensitive crosslinker and polyethylene glycol-based crosslinkers with different topologies we engineered hydrogels with possibilities to control the properties of the cellular microenvironment. Hydrogels were prepared by conjugating the cyclooctyne bicyclo[6.1.0]nonyne (BCN) to hyaluronic acid and crosslinked utilizing degradable and non-degradable crosslinkers in the presence and absence of cyclic RGD (cRGD) as cell-adhesive ligand. Primary fibroblasts were cultured in hydrogels with different stiffness, plasticity, degradability, and amount of cRGD. We identified that cells encapsulated in stiffer hydrogels (G´ ≈ 0.7 kPa) in the presence of cell-adhesive ligand showed an extended morphology, irrespectively of degradability. However, cellular extensions were longer and more defined in degradable compared to non-degradable hydrogels. This effect was further pronounced in hydrogels with shorter relaxation time. In softer hydrogels (G´≈ 0.3 kPa) the extensions were less pronounced. In addition, cells cultured in hydrogels in the absence of cRGD showed a rounded morphology in both soft and stiff conditions. In conclusion, these modular and tunable hydrogels are promising candidates for design of microenvironments to control cell behavior and morphology.

Abstract **#2169**

MODEL-BASED PREDICTIVE CONTROL METHOD FOR MESENCHYMAL STEM CELL EXPANSION: CONTROLLING CELL GROWTH BY PREDICTING MEDIUM REPLACEMENT STRATEGIES IN REAL-TIME

Janne Reynders[P][1], Kathleen Van Beylen[1], Ioannis Papantoniou[2,3,4], Jean-Marie Aerts[1]

[1] *Measure, Model & Manage Bioresponses (M3-BIORES), KU Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium*

[2] *Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Onderwijs en Navorsing 1, Herestraat 49, 3000 Leuven, Belgium*

[3] *Skeletal Biology and Engineering Research Centre, Onderwijs en Navorsing 1, Herestraat 49, 3000 Leuven, Belgium*

[4] *Institute of Chemical Engineering Sciences, Foundation for Research and Technology – Hellas (FORTH), 26504 Patras, Greece*

Corresponding author's email: janne.reynders9@gmail.com

Cell expansion processes are essential to acquire the high amount of cells needed to do clinical research towards cell-based regenerative applications[1]. These bioprocesses are highly variable, risking to overfeed cells when standard protocols are followed[2]. This work proposes novel, individual feeding strategies for bioprocesses in tissue flasks, using lactate values measured during the culture process as an indication for cell growth. The feeding strategies are generated by a model-based predictive controller in MATLAB R2019b. As a reference, human progenitor cells were cultured for 8 days using 4 different, fixed medium replacement strategies, each performed twice. Dynamic regression models were fit with an average accuracy of 94.43% ± 0.04%. The controller was tested by performing simulations with this reference data, resulting in an average accuracy of 97.35% between measured and predicted lactate values. Next, the controller was implemented in real-time during new culture experiments. The amount of medium replaced was based on what the controller predicted, using data from the reference experiments as a trajectory. A first experimental run used the exact lactate values from the reference experiment as a trajectory, resulting in an average accuracy of 10.41% ± 0.08% between reference data and real-time lactate measurements. A second run used rates with which the lactate values increased during the reference experiment as a trajectory, giving an accuracy of 92.78% ± 0.04% between real-time lactate measurements and reference. Using rates of lactate as a reference is therefore a promising way to control cell growth in real-time through predicted, individualized feed strategies.

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Abstract **#2170**

CANCER ORGANOTROPISM-ON-A-CHIP

Molly Shen[P][1], Alia Alameri[1], Andrew Tan[1], Emilie Solymoss[2,3], Grant Ongo[1], Sebastien Tabaries[2,3], Peter Siegel[2,3], David Juncker[1,4]

[1] *Department of Biomedical Engineering, McGill University*

[2] *Rosalind and Morris Goodman Cancer Research Centre, McGill University*

[3] *Department of Medicine, McGill University*

[4] *McGill Genome Centre, McGill University*

Corresponding author's email: molly.shen@mail.mcgill.ca

Decoding the complex biological network within the tumor microenvironment (TME), which consist of spatiotemporally-regulated cell-cell and cell-matrix interactions, is crucial in advancing our understanding of tumor progression and metastasis. Furthermore, the TME secretome remains a key determinant in metastatic organotropism by priming the pre-metastatic niche via signaling proteins and extracellular vesicles (EVs). Thus, an intricate 3D co-culturing system enabling precise tissue localization of multiple cell types while allowing discrete sampling without disturbing the system's biochemical gradients is needed to recapitulate the dynamic interactions within the TME.

We developed an organ-on-a-chip device for co-culturing spheroids of MDA-MB-231 triple-negative breast cancer cell line, or its brain-, bone-, lung-, and liver-organotropic subpopulations, with up to four relevant TME stromal cell types. Organ-native fibroblast, endothelial, and immune cell types were seeded in the side chambers surrounding the spheroid chamber, mimicking cancer-stromal configurations observed in vivo. Conditioned media were sampled from chamber-specific open wells and profiled for secreted proteins and EVs throughout the experimental time-course using antibody microarrays. Our preliminary data suggested that secretomic crosstalk between breast cancer spheroids and stromal cells differed depending on metastatic organotropism. Notably, various integrin expressions in breast cancer-derived EVs were unique among lung-, brain- and bone-organotropic populations. Additionally, confocal live-cell imaging suggested that migration patterns of the spheroid varied depending on its organotropism and the presence of co-cultured stromal cell types.

In conclusion, our platform opens up potential avenues for bridging our understanding of the highly dynamic TME secretome and its heterogenous effects on tumor-stromal migration and metastatic organotropism.

Abstract **#2171**

COLLAGEN-TANNIC ACID SPHEROIDS FOR B-CELL ENCAPSULATION FABRICATED BY 3D BIOPRINTING

Laura Clua Clua Ferré[1]
[1] *FUNDACIÓ INSTITUT DE BIOENGINYERIA DE CATALUNYA*
Corresponding author's email: lclua@ibecbarcelona.eu

Type 1 Diabetes Mellitus is an autoimmune disease characterized by an insulin deficiency caused by pancreatic β-cell destruction, which leads to persistent high blood glucose levels [1]. There is an urgent need for more efficient and cost-effective strategies to ensure physiological insulin production that responds to blood glucose levels. This study has developed a high-throughput and low-cost 3D bioprinting methodology for producing insulin-secreting spheroids through encapsulation of β-cells in a single-step procedure. The reduced spheroid volume and the increment of surface-area-to-volume ratio could improve the diffusion distance compared to large volumes and decrease time response to blood glucose changes. We used collagen as a biocompatible encapsulation material which provides a physiological environment for β-cells. To improve the mechanical stability, we crosslinked the collagen hydrogel with Tannic Acid (TA), which interacts with the collagen chain-forming hydrogen bonds [2]. Additionally, TA possesses pleiotropic effects, such as anti-inflammatory and anticancer properties [3].

This protocol allows customizing the size of the spheroids with extremely low variability, minimizing cell damage during the encapsulation process. Specifically, we show that collagen crosslinked with TA hydrogels present easier handling and far more resistance to collagenase degradation. Furthermore, the stiffness increases in time and dose-dependent manners. Importantly, encapsulated β-cells do not escape from the collagen hydrogels crosslinked with TA, and insulin secretory function is sustained over time.

In conclusion, the present study provides a foundation toolset to generate cell-laden spheroids by 3D bioprinting, which can be helpful for future advanced functional studies.

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Abstract **#2172**

GITBOX: A MINIATURE MULTI-BIOREACTOR SYSTEM FOR STABLE AND REPRODUCIBLE IN VITRO INVESTIGATION OF GUT MICROBIOME

Zijie Jin[1,2], Andy Ng[1,2], William Jogia[1,2], Corinne F. Maurice[3], David Juncker[1,2]

[1] *McGill Genome Centre, McGill University, 740 Doctor Penfield Avenue, Montreal, QC, H3A 0G1 Canada*

[2] *Department of Biomedical Engineering, McGill University, Montreal, Quebec H3A 2B4, Canada*

[3] *Department of Microbiology and Immunology, McGill University, Montreal, QC H3A2B4, Canada*

Corresponding author's email: zijie.jin@mail.mcgill.ca

The gut microbiota has been increasingly recognized in its role for human health and disease(1). In vitro fermentation systems that model various parts of human GI tract allow for the investigation of microbial communities with precise control of various physiological parameters while decoupling interference from the human host. Current systems, such as the Simulator for Human Intestinal Microbial Ecosystem (SHIME)(2) and Robogut(3), while effective at propagating and stabilizing complex microbiota communities, are large in footprint, lack multiplexing and of low experimental throughput. Alternatives which address these shortcomings are often reliant on expensive and delicate lab equipment which hinders lab replication, such as the Mini Bioreactor Arrays (MBRA) system(4). Here, we present GITbox, a low-cost, miniaturized multi-bioreactor system that simulates the human colon microbiome environment with physiologically relevant conditions simultaneously in triplicate bioreactors. The bioreactors had 30ml working volume, were independent of anaerobic chamber and equipped with automated pH, temperature, gas, and fluidic control. We found that from 14-day experiments, GITbox was able to support a stable complex microbiota community from individual fecal samples after only 3-5 days of initial adaptation period. We further characterized GITbox-grown microbiota and found that GITbox retained inter-sample microbiota differences by developing closely related communities distinctive to each donor. GITbox also maintained both minimal variations between replicate reactors and day-to-day variations after stabilization similar to other existing systems and mouse model. Together, we demonstrate that GITbox is an accessible system for studying effect of various xenobiotics and perturbation on gut microbiota with high throughput and multiplex.

Keywords

Microbiome; Miniature; Multiplex

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Abstract **#2173**

PROGRESSION TOWARDS 3D TISSUE ENGINEERED INTRAFUSAL SKELETAL MUSCLE FIBRES

Philip Barrett$_P$[1], Tom J Quick[2,3], Vivek Mudera[1], Darren J Player[1]

[1] *Centre for 3D Models of Health and Disease, Division of Surgery and Interventional Science, Faculty of Medical Sciences, University College London, UK*

[2] *Peripheral Nerve Injury Research Unit, Royal National Orthopaedic Hospital, Stanmore, UK*

[3] *UCL Centre for Nerve Engineering, University College London, London, UK.*

Corresponding author's email: philip.barrett.18@ucl.ac.uk

Intrafusal skeletal muscle fibres (Bag and chain) are a specialised population found within the muscle spindle and have a mechano-sensory capacity, forming a key component of proprioceptive function. Dysfunction of intrafusal fibres is linked with many neuromuscular diseases and injury. To-date, progress towards generating de novo intrafusal fibres for studies of development, function and disease are limited to basic two-dimensional cultures. Neuregulin isoform 1 (Nrg-1) initiates differentiation in vivo and has been the focus of in vitro literature. C2C12s in 2D respond to Nrg-1, with some prominent features of intrafusal skeletal muscle, but with limited expression of putative intrafusal specific proteins. The capacity for C2C12 Intrafusal specific differentiation in a 3D tissue engineered model is undetermined, forming the rationale for this study.

Collagen I, Matrigel and cell mixtures cast into 3D printed moulds, were supplemented every 48hrs with +/-Nrg-1 (100ng/mL) during differentiation. Intrafusal bag myotubes were morphologically assigned from fluorescent micrographs. To elucidate mechanisms underpinning morphological differences, gene (RT-qPCR) expression of key transcription factors, myogenic regulatory factors, and myosin heavy chains (MyHCs) were analysed. Maximal twitch and tetanic forces were measured following electrical field stimulation using a force transducer system.

Ongoing results indicate Nrg-1 supplementation increases intrafusal bag myotube differentiation. MyHC expression was altered in nearly all isoforms, suggesting specificity towards a distinct phenotype comparable to that observed in 2D, but with a shift towards mature isoforms. There were no identifiable increases in putative intrafusal specific genes as characterised in vivo. Functional assessments and metabolic assays are currently being undertaken.

Keywords

Proprioception; Intrafusal; Spindle

Abstract **#2174**

PREPARATION OF MACROPHAGE ADHERING PARTICLES FOR IMMUNOLOGICAL EVALUATION OF BIOMATERIALS

Shota Toda[P][1], Yoshihide Hashimoto[1], Tsuyoshi Kimura[1], Akio Kishida[1]

[1] *Tokyo Medical and Dental University*

Corresponding author's email: toda.mbme@tmd.ac.jp

Recently, macrophage polarization, which expresses pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes, is focused because it strongly related to chronic disease and biomaterial compatibility. In order to evaluate an immunological property of biomaterial, macrophage polarization is assessed in vivo and in vitro. Generally, for in vitro evaluation of macrophage polarization, macrophage was detached from culture dish enzymatically and physically and subjected to macrophage polarization analysis. However, it has been reported that these treatments strongly affect the macrophage polarization ability due to the cell membrane damage. Therefore, in this study, we proposed a new method of macrophage polarization evaluation, which is utilization of macrophage adhering macroparticles, without the cell detachment process. THP-1 cell was used as a model of macrophage. Polystyrene macroparticles were used. THP-1 adhering particle was prepared by rotation culture of mixture of THP-1 and particle stimulating with phorbor-myristate-acetate. The THP-1 cells were adhered on particle uniformly with 30-40 cells/particle. The macrophage polarization ability on particle stimulating with LPS/IFNγ and IL-4/IL-13 was investigated by using RT-PCR analysis of IL-1β and MRC1, which were markers of M1 and M2, respectively. The high expressing of IL-1β and MRC1 on particle was showed compared to macrophage treated with detachment process. From these results, we have successfully developed a new method of macrophage polarization using the macrophage adhering particles.

Keywords

Biomaterial property; Macrophage polarization

Abstract **#2175**

DEVELOPING A SELF-ASSEMBLING PEPTIDE HYDROGEL-ASSISTED COMBINATION THERAPY FOR BRAIN REPAIR FOLLOWING ISCHAEMIC STROKE

Roxana Sava[P][1], Raymond Wong[1], Camilo Albornoz[1], Cosimo Ligorio[2,3], Alberto Saiani[2,3], Jeffrey Penny[1], Stuart Allan[1], Emmanuel Pinteaux[1]

[1] *Faculty of Biology, Medicine and Health, The University of Manchester*

[2] *Manchester Institute of Biotechnology, The University of Manchester*

[3] *Faculty of Science and Engineering, The University of Manchester*

Corresponding author's email: oana-roxana.sava@manchester.ac.uk

With no available treatments for brain tissue repair and functional recovery in patients with lost or impaired neurological functions, stroke therapy remains an important unmet clinical need. The lack of structural support in the stroke cavity and insufficient endogenous tissue repair dramatically limit brain functional recovery after stroke. Novel regenerative therapies aim to use soft hydrogels that can offer structural support and deliver pro-regenerative therapeutic agents whilst improving their half-life [1,2]. We propose a novel combination therapy based on a self-assembling peptide hydrogel (SAPH), the anti-inflammatory and microglial modulator tumor necrosis factor-alpha stimulated protein-6 (TSG-6) [3], and the angiogenic and neurotrophic vascular endothelial growth factor (VEGF). Here, we show that following intra-cerebral injection in healthy mice, RGD/IKVAV-PeptiGel-Alpha2 hydrogel did not induce microglial activation or astrocytic scarring, suggesting its biocompatibility with the brain tissue. Furthermore, the hydrogel released VEGF in a sustained manner in vitro. These findings support the use of RGD/IKVAV-PeptiGel-Alpha2 as a temporary scaffold for brain repair and VEGF release system for angiogenic applications following brain injury. In parallel, we have tested the reparative properties of TSG-6 administered sub-acutely into the stroke infarct. Results have shown a trend toward improved wellbeing and decreased microglial activation, as well as signs of angiogenesis at 28d post-stroke. Ongoing work is testing the efficacy of TSG-6 and VEGF combination assisted by the RGD/IKVAV-PeptiGel-Alpha2 delivery system to enhance tissue repair and promote functional recovery after stroke.

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Abstract **#2176**

EFFECT OF ELECTRICAL AND MECHANICAL STIMULATIONS ON CONTRACTILITY AND MYOKINE SECRETION OF TISSUE-ENGINEERED SKELETAL MUSCLE

Toshia FUJISATO$_P$[1]

[1] *Osaka Institute of Technology, Osaka, JAPAN*

Corresponding author's email: toshiya.fujisato@oit.ac.jp

Tissue engineering can be applied to three-dimensional skeletal muscle model for physical exercise. In this study, the tissue-engineered skeletal muscle was cultured under various electrical and mechanical stimulations and examined its contractility and myokine secretion.

Two artificial tendons were introduced at both ends of our engineered muscle. The two tendons were hold with stainless steel pins placed 12 mm apart before cell seeding. The C2C12 myoblasts embedded in collagen gel was added between and on the surface of the two tendons. After gelation of the cell suspension, the constructs were cultured in growth medium of high-glucose DMEM with 10% FBS for 2 days. Then, they were cultured in differentiation medium of high-glucose DMEM with 7% horse serum with various electrical pulse stimulation or passive mechanical stretch movements. After the stimulation, the contractile force was measured by micro load cell and the amount of secreted IL-6 in the culture medium was determined by ELISA.

The isometric twitch force of the tissue-engineered skeletal muscle cultured with electrical pulse of 5V, 2msec, and 0.5Hz was remarkably higher than that of without stimulation up to 7 days. However, there was no difference when the constructs were cultured more than 14 day with electrical stimulation. In addition, the amount of IL-6 secreted from stimulated skeletal muscle was larger than that of without stimulation.

These results suggest that tissue-engineered skeletal muscle may be applied to physical exercise model in vitro.

Abstract **#2177**

ADDITIVE MANUFACTURING OF POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE)/POLY(D,L-LACTIDE-CO-GLYCOLIDE) BIPHASIC SCAFFOLDS FOR BONE TISSUE REGENERATION

Gianni Pecorini[P][1]

[1] *University of Pisa - Department of Chemistry and Industrial Chemistry*

Corresponding author's email: gianni.pecorini@phd.unipi.it

Bone tissue lesions are among the most common injuries of the human body. The increased need for bone replacement becomes a key scientific, socio-economic, and clinical challenge. Tissue Engineering-based strategies are providing reliable therapeutic tools for healing damaged bone tissue [1]. Polyhydroxyalkanoates are bio-polyesters whose biocompatibility, biodegradability, sustainability, good processing versatility and mechanical properties make them unique scaffolding polymer candidates for Tissue Engineering [2]. The development of new materials suitable for advanced biofabrication techniques, such as Additive Manufacturing (AM), are of primary importance for the advancement of Tissue Engineering. The formation of polymeric blends represents a useful strategy to develop new scaffolds that can mimic the properties of the target tissue [3]. In this contribution scaffolds from polymeric blends consisting of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(D,L-lactide-co-glycolide) (PLGA) have been fabricated employing a solution-extrusion AM technique, referred to as Computer-Aided Wet-Spinning. Scaffold's fibers were constituted by a biphasic system formed by a continuous PHBV matrix and a dispersed PLGA phase which assumed a microfibrillar morphology. The influence of blend composition on morphological, physical-chemical and biological properties of the scaffolds was evaluated. In particular, increasing the content of PLGA in the starting solution resulted in an increase of the pore size, the wettability, and the thermal stability of the scaffolds. Overall, the biological evaluation indicated the suitability of the scaffolds to support murine preosteoblast cells colonization as well as their capability to promote cell differentiation towards an osteoblastic phenotype, highlighting better results for scaffolds richer in PLGA.

Keywords

Polyhydroxyalkanoates; Additive Manufacturing; Biphasic Scaffolds

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Abstract **#2178**

NOVEL DERMAL TEMPLATE PRODUCTION FOR EFFECTIVE BURNS WOUND HEALING

Weng Wan Chan[P][1], David Chen Loong Yeo[1], Sayan Chakraborty[2], Gibson Chin Yuan Soo[3], Wai Tat Kerk[3], Ying Ying Wu[1], Boon Keng Lok[3], Deepak Choudhury[1], Norris Ray Dunn[4], May Win Naing[3]

*[1] Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, Centros, Singapore 138668, Singapore*

*[2] Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), 61 Biopolis Dr, Proteos, Singapore 138673, Singapore*

*[3] Singapore Institute of Manufacturing Technology (SIMTech), Agency for Science, Technology and Research (A*STAR), 2 Fusionopolis Way, Innovis, Singapore 138634, Singapore*

[4] Lee Kong Chian School of Medicine, Nanyang Technological University (NTU), 11 Mandalay Road, Clinical Sciences Building, Singapore 308232, Singapore

Corresponding author's email: chan_weng_wan@bti.a-star.edu.sg

Treating deep-second degree burns, and third-degree burns requires debridement of nonviable tissue, attainment of homeostasis followed by skin graft application to assist wound closure. The severity of burns wounds impairs blood supply, giving rise to poor wound bed vascularization resulting in impaired regeneration and diminished grafting. To address this issue, the wound bed is prepared using a dermal substitute to re-vascularize the dermis layer prior to graft application [1]. While existing products such as Integra® prepare the wound bed for skin grafting, a relatively long period of 15 to 20 days is required for neodermis formation and wound bed re-vascularization [2]. Reducing the time taken for re-vascularization and neodermis formation results in better wound healing outcomes.Within the 'additive manufacturing for biological materials' (AMBM) program that seeks to biomanufacture the next-generation of burns wound healing technology, we are developing processes for producing novel biomimetic dermal templates which improve wound healing via incorporation of pro-vascularization and pro-regenerative additives.Herein, we demonstrated that collagen scaffold produced via manual fabrication have pore sizes matching the target range (20-125µm) and attain 32% crosslinking following glutaraldehyde (GA) treatment. Successful incorporation of bio-additives into scaffolds was demonstrated and further shown to be biocompatible with variable degradation rates during validation in the Chick Chorioallantoic Membrane (CAM) assays. Following manual fabrication, scaled-up manufacturing techniques were used to produce multiple 2.5cmx2.5cm scaffolds to demonstrate scale-up production potential. These collaborative efforts by the program show promising results in developing novel dermal template that improve healing in severe burn wounds.

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Abstract **#2179**

THE IL-23R/IL-17 SIGNALING CASCADE IN ACUTE ACHILLES TENDON RUPTURES AND CHRONIC TENDINOPATHIES

Christiane Gäbel[P][1,2], Anja Kühl[3], Tobias Gehlen[4], Serafeim Tsitsilonis[4], Uta Syrbe[5], Britt Wildemann[6,7], Franka Klatte-Schulz[6], Birgit Sawitzki[1]

[1] *Institute of Medical Immunology, Charité-Universitätsmedizin Berlin, Germany*

[2] *Berlin-Brandenburg School for Regenerative Therapies, Germany*

[3] *IPATH.Berlin – Core Unit Immunopathology for Experimental Models, Charité-Universitätsmedizin Berlin, Germany*

[4] *Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin, Germany*

[5] *Department of Gastroenterology, Infectiology and Rheumatology, Charité - Universitätsmedizin Berlin, Germany*

[6] *Julius Wolff Institute, BIH Center for Regenerative Therapies, Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Germany*

[7] *Experimental Trauma Surgery, Universitätsklinikum Jena, Germany*

Corresponding author's email: christiane.gaebel@charite.de

In Spondyloarthritis (SpA)-associated Achilles tendon enthesitis, IL-23 receptor (IL-23R) expressing γδ-T-cells that secrete cytokines of the IL-17 family were described to play a driving pathogenic role.[1] We investigated whether the IL-23R/IL-17 signaling cascade also contributes to the development of non-SpA-associated tendon pathologies.

Achilles tendon biopsies from acute ruptures (n=39) and chronic tendinopathies (n=17) were analyzed immunohistologically and by flow cytometry to prove the presence and identify the cellular source of IL-23R, IL-17A, IL-17F, IL-22. Moreover, hematoma aspirate was harvested from the Achilles tendon rupture side and analyzed for IL-23R/IL-17 signaling mediators on protein level.
Immunohistology revealed the presence of IL-23R+ and IL-17A/F+ cells in acute ruptures and chronic Achilles tendinopathies. Interestingly, 100% of the chronic specimens contained IL-23R+ cells, whereas only 42.9% of acute ruptures showed IL-23R+ cells. Furthermore, a higher percentage of the chronic specimens showed IL-17A/F+ cells (46.7%) compared to the acute ruptures (20%). Preliminary flow cytometry data identified tenocytes as possible source of IL-17F and IL-22, but not of IL-17A expression. The analysis of hematoma aspirate revealed elevated concentrations of IL-23, IL-17F and IL-22 in 4 of 24 patients with acute Achilles tendon rupture.

We demonstrate the presence of mediators of the IL-23R/IL-17 signaling axis in non-SpA-associated Achilles tendon pathologies with differences between acute ruptures and chronic tendinopathies. Compared to IL-17A as trigger in the SpA-associated enthesitis model, we propose that IL-17F might drive non-SpA Achilles tendon pathologies.[1] The results of the project contribute to a better understanding of underlying pathomechanisms of acute ruptures and chronic tendinopathies.

Keywords

Achilles Tendon pathology; IL-23R/IL-17 signaling

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Abstract **#2180**

3D PRINTING OF CITRATE-BASED BIOMATERIALS FOR FABRICATION OF BIODEGRADABLE 3D SCAFFOLDS

Vahid Karamzadeh$_P$[1], Ioannis paschalidis[1], Scott campbell[2], Milica Radisic[2], David Juncker[1]
[1] *Biomedical Engineering Department, McGill University, Montreal, Quebec, Canada*
[2] *Institute of Biomaterial and Biomedical Engineering, University of Toronto, Toronto, Canada*
Corresponding author's email: vahid.karamzadeh@mail.mcgill.ca

Tissue-engineered scaffolds fabricated through conventional techniques such as lithography, and stamping are limited in their geometrical complexity, fabrication speed, and automation capacity [1, 2]. To overcome these limitations, 3D printing based on Digital Light Processing (DLP) technology has been gaining popularity, however, there is a lack of suitable photo-biopolymers to exploit the potential of light-based 3D printers [3, 4]. This study optimizes a photo-crosslinkable, biocompatible and biodegradable elastomer - poly(octamethylene maleate (anhydride) citrate) (POMaC) [1, 5, 6, 7] for DLP 3D printing through careful consideration of the interplay between printing parameters, and ink formulation, such as monomer ratio and the use of an appropriate photoinitiator. The POMAC prepolymer was premixed with the Irgacure-819 photoinitiator, and polyethylene glycol dimethyl ether (PEGDM), a material with viscosity as low as 57 cPa, serving both to reduce the viscosity and enable the formation of nanopores. POMaC was successfully fabricated into intricate free-form lattices with a sub-millimetre resolution, as well as semi-embedded features down to a width of 70 µm. The use of the multi-exposure mode presented here allows rapid selection of an appropriate exposure time. The optimized exposure time for a 20 µm layer of premixed polymer is 55s, which is considerably faster than the 4-minute crosslinking time required in manual stamping of the same material [1]. By combining the previously demonstrated biocompatibility, biodegradability and mechanical versatility [1, 5, 7, 8, 9] with the printability achieved in this study, POMaC serves as a paradigm for biomaterial design in soft organ-on-a-chip engineering.

Keywords
3D printing; Biomaterials; 3D scaffolds for TE applications

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Abstract **#2181**

SMART PIEZOELECTRIC GRAPHENE REINFORCED POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXY VALERATE) NANOFIBROUS SCAFFOLD FOR CARTILAGE REGENERATION

Namdev More$_P$[1], Abhishek Roy[2], Hemant Kumar[2], Akshay Srivastava[1], Govinda Kapusetti[1]

[1] *Department of Medical Devices, National Institute of Pharmaceutical Education and Research, Ahmedabad, Gandhinagar India.*

[2] *Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Ahmedabad, Gandhinagar India*

Corresponding author's email: more.namdev.b@gmail.com

Cartilage regeneration remains a formidable challenge because of its harsh physiological environments, such as avascularity and aneurality. Piezoelectric biomaterials emerged as a promising source of scaffolds for cartilage repair and regeneration. We fabricated a piezoelectric smart electrospun scaffold from biologically originated Poly(3-hydroxybutyrate-co-3-hydroxy valerate) (PHBV) polymer reinforcement with carbon-based nano-material such as Graphene oxide (GO) and Amino-graphene (AG). The bead-free and smooth nanofibers were fabricated by electrospinning technique and characterized with FE-SEM and AFM techniques. The spectroscopic and thermal studies reveal that there is no significant interaction between reinforcing agent (GO and AG) and polymer. The scaffolds with GO and AG demonstrated improved mechanical and piezoelectric properties against the pristine PHBV. The polarised scaffolds in presence of GO and AG evident for better ECM production and chondrogenic marker expression as compare to PHBV. The developed piezoelectric scaffold will also be characterized under dynamic loading conditions to demonstrate the effect of piezoelectricity in tissue formation. The developed scaffold was also characterized in vivo rat osteochondral defect model where PHBV-AG showed excellent cartilage tissue integration and regeneration compared to PHBV and PHBV-GO. The developed piezoelectric scaffold will also be characterized under dynamic loading conditions to demonstrate the effect of piezoelectricity in tissue formation. The developed scaffold will also be characterized in vivo rat osteochondral defect model. Keywords: Piezoelectricity, Cartilage regeneration, Poly(3-hydroxybutyrate-co-3-hydroxy valerate), Graphene, Electrospinning, polarisation.

Abstract **#2182**

CHALLENGES IN 3D CELL CULTURE: VALIDATION OF CELL VIABILITY ASSAYS IN 3D BRAIN TUMOUR MODEL

Mahsa Vaezzadeh$_P$[1], Benjamin Heng[1], Anna Guller[1,2,3], Gilles J. Guillemin[1], Annemarie Nadort[3,4]

[1] *Department of Biomedical Sciences, Macquarie University, Sydney, Australia*

[2] *The Graduate School of Biomedical Engineering, University of New South Wales, Sydney, Australia*

[3] *ARC Centre of Excellence for Nanoscale BioPhotonics, Macquarie University, Sydney, Australia*

[4] *Department of Physics and Astronomy, Macquarie University, Sydney, Australia*

Corresponding author's email: mahsa.vaezzadeh@hdr.mq.edu.au

Background: 2D Preclinical assays have largely failed to effectively predict the efficacy of anticancer drugs. Moving to 3D models, one of the challenges is how to assess drug efficacy. Viability assays are typically used in low cell density 2D cultures and may not produce the same results in 3D cultures.

Aim: Our aim is to validate the common viability assays in three different models: conventional 2D culture, matrigel-based 3D culture and an organ-specific 3D brain tumour model recently developed by us.

Methods: Identical U251 cell numbers were plated in 2D and were mixed with matrigel to form a 3D culture model. Organ-specific 3D scaffolds were developed by decellularizing animal brain tissue followed by seeding with U251 cells. We tested the performance of four different viability assays (MTT, Alamar Blue, ATP, LDH) on three different culture models and calibrated the assay output versus known cell numbers by counting and DNA quantification.

Results: In all of our selected cell viability assays, the assay output was higher in 2D samples compared with matrigel samples containing the same number of cells. In MTT assay by increasing the reagent concentration the difference between assay reading in 2D and matrigel samples became less which might indicate that there is a decreased reagent availability to the cells grown in Matrigel.

Conclusions: Most protocols for viability assays that are validated for 2D cell culture are not (directly) applicable to 3D models, however, optimization and calibration can make them relevant for 3D cell culture.

Keywords

3D cell culture; Extra cellular matrix; Cell viability assay

Abstract **#2183**

IMMUNOMODULATORY AND ELECTRO-CONDUCTIVE GRAPHENE-CROSSLINKED-COLLAGEN SUPERMACROPOROUS BRIDGE FOR ACCELERATED SPINAL CORD REGENERATION

Gopal Agarwal$_P$[1], Abhishek Roy[2], Hemant Kumar[2], Akshay Srivastava[3]

[1] *Department of Biotechnology, National Institute of Pharmaceutical Education and Research, Ahmedabad, Gujarat, India-382355*

[2] *Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Ahmedabad, Gujarat, India-382355*

[3] *Department of Medical Device, National Institute of Pharmaceutical Education and Research, Ahmedabad, Gujarat, India-382355*

Corresponding author's email: gopalniperbt@gmail.com

Inability of neurons to spontaneously proliferate and prevalence of neuro-inflammation at the site of injury are the major barrier for spinal cord injury treatment. To overcome this issues, we have fabricated an electroconductive and immunomodulatory graphene-crosslinked-collagen cryogels. The fabricated cryogels showed an electro-conductivity of 3.8±0.2 m Siemens/cm, and supports the growth and proliferation of bone marrow derived mesenchymal stem cells. Besides this, the fabricated graphene collagen cryogels had well interconnected porous structure, as evident by FESEM and Cryo-FESEM analysis. The incorporation of graphene has increase the mechanical strength of cryogels, and also have resulted in increase of collagen fiber alignment as analyzed using Raman spectroscopy. Further, ex vivo culture of spinal cord slice culture study demonstrated the cryogel potentials in controlling astrocytes reactivity and promoting neuronal development upon external electric stimulus. Additionally, in vivo implantation in rat's hemi-sectioned model at thoracic level T9-T11 demonstrated the animals with cryogels showed faster recovery from paralysis within 7-10 days of implantation. The cryogels has also shown the capability in polarization of microglia into M2 phenotype and showed higher neuronal network formation after 28 days of implantation as evident by higher expression of β-tubulin III and neurofilament 200. Upon investigating the possible mechanism, we found the role of STAT3, and increased extra-cellular matrix development in controlling the neuro-inflammation and fostering the axonal development at the site of injury respectively.

Abstract **#2184**

VASCUSKIN – ENGINEERING A VASCULARIZED SKIN EQUIVALENT FOR SENESCENCE STUDIES

Barbara Bachmann[1,2,3], Wolfgang Holnthoner[2,3], Johannes Grillari[2,3], Heinz Redl[1,2,3], Mikolaj Ogrodnik[1,2,3]

[1] *Ludwig Boltzmann Research Group Senescence and Healing of Wounds, 1200 Vienna, Austria*

[2] *Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in the AUVA Research Centre, 1200 Vienna, Austria*

[3] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*

Corresponding author's email: barbara.bachmann@trauma.lbg.ac.at

A vascularized and perfusable in vitro model of human skin would facilitate skin aging and tissue injury research. A critical player in both processes is cellular senescence, a state of irreversible cell cycle arrest. Senescent cells arise in injured tissues and have been found causally involved in accelerating the healing of acute wounds but impeding chronic wound closure. Current experimental dermatology models investigating the impact of senescence on tissues mainly involve animal models due to the skin's cellular heterogeneity, complex anatomy, and a lack of suitable in vitro models. However, ethical concerns, long experimental times, and restricted experimental flexibility limit the applicability of these in vivo models for wound-healing associated senescence studies. As a solution, we engineered a vascularized 3D full-thickness in vitro skin model including epidermis, dermis, and microvasculature (vascuSKIN) to create an accessible and standardized in vitro skin model. Our approach mimics human skin in vitro, allowing us to gain knowledge on cellular-level wound healing processes impossible to acquire in animal models. Our model uses a customized vascuSKIN device that enables (a) the connection of the vasculature to external pumps and (b) the elevation of the cultures to the air-liquid interface to form a stratified epithelium. The vascuSKIN recreates skin by including a dermis and an epidermis cultivated at the air-liquid interface that consists of the essential cell types associated with senescence-associated processes in wound healing. We will apply the developed full-thickness vascularized skin equivalents to study wound healing and the role of associated senescent cells in vitro.

Abstract **#2185**

ENGINEERING NANO-ARCHITECTED SURFACES TO STEER SOFT AND HARD TISSUE INTERACTIONS ON TISSUE LEVEL DENTAL IMPLANTS

Judith Ng[P][1,2], Martin Matter[1], Kerda Keevend[1], Stefanie Guimond[2], Markus Rottmar[2], Inge Herrmann[1,2]

[1] *ETH Zurich*

[2] *Empa*

Corresponding author's email: judithng1@gmail.com

Dental implant failures arise from poor integration of the implant with surrounding soft and hard tissues. Osseointegration and periointegration are crucial to their prevention. A persistent challenge is the inconsistency between tissue surrounding an implant and natural periodontal tissue around natural teeth. This increases vulnerability to peri-implant diseases, affecting soft and hard tissue integration and highlighting the need for implants with improved osseointegration and periointegration potential. Implant surface nanostructures promote osseointegration; however, designs which improve the soft tissue response have yet to be identified.

We present novel nanoparticle coatings applied to surfaces in a single step technique driven by liquid-feed flame spray pyrolysis, which uniquely permits the construction of bespoke nanoparticle architectures from a breadth of inorganic precursors. This highly scalable technique has the potential to create multi-layer nanostructures of differing thicknesses, diverse patterns, and pore sizes – qualities as diverse as the inorganic building blocks of which these nanoparticle layers are composed.

Novel nanoparticle-based coatings with bioactive inorganic architectures can enhance osseointegration, reduce the inflammatory response, and possess exemplary process versatility and stability, permitting precise control over the synthesis process, and so too, evaluation of the concomitant soft tissue bioresponse.

Keywords

nanoparticle; dental implant

Abstract **#2186**

INVESTIGATION OF NEUROVASCULAR EFFECTS OF MARINE-DERIVED MOLECULES IN 3D MICRO FRAME CO-CULTURE MODEL

Emel Sokullu[P][1,2], Gulgun Sengul[3], İrem Polat[4], Ferhat Can Ozkaya[5], Mohamed-Farid Lahloubd[6], Weaam Ebrahimd[6]

[1] *Koç University School of Medicine*

[2] *Zetamatrix A.S.*

[3] *Koç University*

[4] *Izmir Katıp Celebi University*

[5] *EBILTEM, Ege University Science and Technology Centre*

[6] *Faculty of Pharmacy, Mansoura University*

Corresponding author's email: esokullu@ku.edu.tr

Tissue engineering is one of the most recent studies to make modeling in order to optimize the tissues that cannot be adequately structurally and functionally.

In this study, gelatin methacryloyl (GelMA) synthesis was performed. GelMA polymerization was performed with ultraviolet ray (UV) using photomask with the device designed by our team. Polymerized hydrogel human umbilical vein endothelial (HUVEC) and neuroblastoma (SH-SY5Y) cells were encapsulated. The effects of marine-derived 'Citreohybridonol' molecule doped into GelMA in different ratios on the co-cultured cells were investigated.

Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), atomic force microscopy (AFM) analyzes were performed to characterize the synthesized GelMA polymer. Cell viability was observed by methylthiazole diphenyl tetrazolium (MTT) and hydrogen peroxide (H2O2) for 14 days. Numerical data obtained from viability analysis were analyzed by GraphPad Prism 8 two-way ANOVA and Tukey test as post hoc. Immunohistochemical staining was performed by using Angiopoietin 1 polyclonal antibody for HUVEC cell line and Nestin antibody for SH-SY5Y cell line in order to observe the molecular effect.The obtained images were found to support cell viability analysis.

In all analyzes, the marine-induced "Citreohybridonol" molecule did not have a negative effect on the HUVEC cell line. In SH-SY5Y cell line, it was found to have a negative effect on cell viability.

Keywords: HUVEC, SH-SY5Y, human umbilical vein endothelium, neuroblastoma, citreohybridonol, gelatin methacrylate, hydrogel, photopolymerization, ultraviolet beam

Abstract **#2187**

BIOFABRICATION OF NANOSTRUCTURED GELATIN FIBER VIA CENTRIFUGAL SPINNING METHOD

Aykut Sancakli$_P$[1], Aysegul Uzuner[1], Fatih Arican[1], Ezgi Ismar[1]

[1] *KCL R&D*

Corresponding author's email: aykutsancakli@iskefeholding.com.tr

In this study, type-I bovine collagen solved in diluted acidic solutions (acidic acid and formic acid) and fed into the centrifugal spinning device to obtain nanofiber formation. The centrifugal spinning device is providing submicron fibers thanks to its rotational movement during the solution feeding. Different feeding ratios and rotational speeds are studied to optimize the process. Obtained nanofiber webs were observed via scanning electron microscopy (SEM). Structural characterization of samples was investigated via FTIR. Fabricated collagen-based nanofibers filtration performance was evaluated in terms of filtration efficiency and air permeability tests. Tests results are pointed that fabricated gelatin nanofibers can be an efficient alternative biomaterial design against Covid-19.

Keywords

Gelatin nanofiber; Mask

Abstract **#2188**

THREE-DIMENSIONAL NEUROVASCULAR CO-CULTURE INSIDE A MICROFLUIDIC INVASION CHEMOTAXIS CHIP

Emel Sokullu[P][1], Levent Cucuk[2], İrem Polat[3], Savas Tasoglu[4,5]

[1] *Koç university*

[2] *Izmir Katip Celebi University*

[3] *Izmir Katıp Celebi University*

[4] *Koç University*

[5] *Zetamatrix A.Ş.*

Corresponding author's email: esokullu@ku.edu.tr

One of the most rapidly emerging tools of nanoscience and nanotechnology is microfluidic chips. With the increase of alternative production techniques and improved capacity of manufacturing devices, it became possible to create microfluidic chips with high resolution and excellent accuracy which can be used for many different fields. In this research, to create a three dimensional cell culture environment, a microfluidic chip was used. To create the three dimensional scaffold structure, Gelatin Methacrylate (GelMA) was synthesized. With using ultraviolet light and photoinitiator a three dimensional hydrogel structure was built. With this method, an alternative and easier strategy, compared to other techniques used to create hydrogels inside of microfluidicchips was proposed.

Chemical compounds of synthesized GelMA was identified with fourier transform infrared spectophotometry (FITR), surface morphology was observed with field emission electronmicroscopy (FESEM), and structural properties was analyzed with atomic force microscopy (AFM). Inside of the produced GelMA hydrogel structure, a neurovascular co-culture was created, and cellular viability of the hydrogel created inside of microfluidic chip was compared with the hydrogel created outside of the chip. Swelling performance of the hydrogel inside of microfluidic chip was observed visually and permeability of this hydrogel was examined with a migration study as a proof of concept.

Results show that synthesized GelMA has desired biomechanical properties with more than 80% cellular viability while being structurally stable, and it was shown that even if its produced inside of microchip, it still has the same biomechanical properties.

Keywords

blood brain barrier

Abstract **#2189**

MICROARRAY PLATFORM FOR HUMAN LUNG ORGANOID LONG-TERM CULTURE AND MANIPULATION

Danielle Baptista$_P$[1], Niloofar Tahmasebi[1], Helene Widowski[2], Fiona Passanha[1], Valisi Stylianidis[3], Kevin Knoops[4], Tim Wolfs[2], Clemens van Blitterswijk[1], Vanessa LaPointe[1], Pamela Habibovic[1], Niki Reynaert[3], Stefan Giselbrecht[1], Roman Truckenmüller[1]

[1] *MERLN Institute, Maastricht University*

[2] *Department of Pediatrics, School of Oncology and Developmental Biology (GROW), Maastricht University*

[3] *Department of Respiratory Medicine, Maastricht University*

[4] *Microscopy CORE Lab Maastricht Multimodal Molecular Imaging Institute (M4I), Maastricht University*

Corresponding author's email: danielle.baptista@maastrichtuniversity.nl

Latest advances in three-dimensional (3D) cell culture technologies have shown outstanding assembling and self-organizing properties of embryonic and adult mammalian stem cells into organoids that replicate key structural and functional properties of organs such as the lungs, hence, becoming an indispensable tool for basic and translational research(1) . Current protocols to generate lung organoids rely on gel-like basement membrane matrices, in which cells assemble and self-organize into multiple randomly “floating” organoids(2). However, these culture methods present some challenges for downstream analysis and are not easily compatible with translational research.

Herein, we propose a platform that consists of an array of micro-wells thermoformed on a polycarbonate film(3), where, in a single film, 289 organoids can be generated. Additionally, the platform enables long-term culture and (real-time) monitoring of lung organoids. This platform surpasses conventional culture methods with a superior observability, traceability and possibility of controlled manipulation of single organoids. Our findings showed that the lung organoids were successfully cultured in the arrayed micro-well platform using established culture protocols and with reduced matrix content for up to two months, and maintained their phenotypical and functional properties. Furthermore, we monitored the development of individual organoids and the fusion of multiple organoids, forming one functional spherical luminal structure. Finally, we demonstrated the individual manipulation of organoids on this platform by luminal microinjection of a dye. These results highlight the potential of the film-based microarray culture platform for gaining in-depth understanding of organoid development in fundamental studies and for employing organoid models in translational research.

Keywords

organoids; microthermoformed array; manipulation

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Abstract **#2190**

"HIGH RESOLUTION 3D BIO-PRINTED SKIN TISSUE MODEL BASED ON PHOTO-CROSSLINKABLE HYDROGELS"

Angela Cirulli$_P$[1,2], Livia Neves Borgheti-Cardoso[1,2], Nuria Torras Andrés[1,2], Jordi Comellas Pujadas[1,2], Gabriele Di Napoli[3], Brigitte Angres[3], Helmut Wurst[3], Elena Martinez Fraiz[1,2,4]

[1] *Institute for Bioengineering of Catalonia (IBEC)*

[2] *The Barcelona Institute of Science and Technology (BIST)*

[3] *Cellendes GmbH*

[4] *Department of Electronics and Biomedical Engineering, University of Barcelona (UB)*

Corresponding author's email: acirulli@ibecbarcelona.eu

Skin is the largest tissue of the human body and represents our daily barrier against the external environment. Nowadays, mimicking the complex skin structure with high spatial resolution is one of the most important challenges of tissue engineering, for skin replacement in transplantations, drug testing and disease modelling (1,2). Here we present a novel approach based on visible-light 3D bio-printing to produce skin tissue models as faithful as possible to the in-vivo structure. Hydrogels scaffolds are the best choice to echo the perfect cell microenvironment due to their biocompatibility and their tunable properties. A Direct Laser Writing system (DLW), equipped with a 405 nm wavelength laser diode and automated translational stages, is used to print photo-polymerizable hydrogels, based on Norbornene thiol-based reactions (3), preserving both mechanical and biochemical properties of cell microenvironment and reproducing skin heterogenous architecture. Different polymer compositions were evaluated in terms of printing morphology and stability. Cell-laden scaffolds with embedded human skin fibroblasts were fabricated and kept in standard culture conditions for several days, showing cell spreading and migration, resulting in high cell viability values. Co-culture systems studies, including both fibroblasts and keratinocytes, containing RGD cell-friendly motifs to promote cell attachment on the scaffolds' surface, have been performed showing cellular compatibility. Furthermore, immunostaining results revealed an enhanced production of extracellular matrix (ECM) proteins compared to single fibroblasts cultures, proving that the combination of photo-crosslinkable hydrogels based on thiol-Norbornene chemistry and co-culture systems provides promising skin-like models.

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Abstract **#2192**

SILKGRAFT, A SILK FIBROIN TUBULAR SCAFFOLD FOR THE REPAIR AND REGENERATION OF SMALL CALIBER BLOOD VESSELS: LONG-TERM IN VIVO STUDY

Antonio Alessandrino[P][1], Marco Biagiotti[1], Giulia Bassani[1], Valentina Vincoli[1], Piergiorgio Settembrini[2], Alberto Settembrini[3], Giuliano Freddi[1]

[1] *Silk Biomaterials srl, Lomazzo, Italy*

[2] *University of Milan, Milan, Italy*

[3] *Department of Vascular Surgery, Fondazione Ca' Granda Ospedale Maggiore, Milan, Italy*

Corresponding author's email: antonio@silkbiomaterials.com

Silk fibroin is an eligible biomaterial for the development of small-caliber vascular grafts for reconstruction of blood vessels [1]. A new multi-layered SF tubular scaffold for vascular grafting (SilkGraft) has been designed and tested in vitro and in vivo. The wall architecture consists of two electrospun layers (inner and outer) and an intermediate textile layer. The latter confers high mechanical performance, while the electrospun layers allow enhancing the biomimicry properties and host's tissues integration of the device. In vitro cell interaction studies demonstrated the biocompatibility of the graft, while in vivo preliminary pilot trials on minipig and sheep allowed assessing the functional behavior of the implanted SF-based vascular graft and to identify the sheep as the more apt animal model for medium-to-long term preclinical trials.
In this study, SilkGraft was used to replace carotid artery in a sheep model (internal diameter = 4.5mm, length = 80mm). Acute performance of the implant was evaluated by Doppler ultrasound and angiography. Chronic performance was evaluated by Doppler ultrasound and/or angiography at Week 4, 26, and 52. Degradation, local tissue effects, and distant systemic effects were evaluated 52 weeks following implantation.
The SilkGraft implantation was successful. After 52 weeks patency of the graft was demonstrated by Doppler ultrasound, angiography, and histopathology. Degradation was moderate and mechanical resistance was guaranteed by the effective integration of SilkGraft by the new endogenous tissues showing also a good endothelialization.
These results demonstrated the opportunity for SilkGraft to be an "off-the-shelf" device for small blood vessel reconstruction.

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Abstract **#2193**

IDENTIFICATION OF MECHANICAL CUES DURING EARLY LIMB BUD DEVELOPMENT USING INDIVIDUAL CELL BASED MODELING

Yvenn Sermeus[P][1,2], Maxim Cuvelier[1,2], Liesbet Geris[2,3], Przemko Tylzanowski[4,5], Bart Smeets[1,2]

[1] *KU Leuven, MeBioS*

[2] *KU Leuven, Prometheus*

[3] *U Liège, GIGA In silico medicine*

[4] *KU Leuven, SBERC*

[5] *Medical University of Lublin, Department of Biochemistry and Molecular Biology*

Corresponding author's email: yvenn.sermeus@kuleuven.be

Mesenchymal condensation is one of the earliest stages of bone tissue development and its a critical step for the resulting biological structure. Many attempts have been made to replicate the condensation process in vitro in order to produce high quality engineered bone tissue. Condensation is not only regulated through chemical signaling but also through mechanical cues. Hence, mechanical stimulation has been suggested as a way to control condensation. Indeed, micro-aggregate experiments show how compression, tension, shear stress and substrate stiffness influence condensation and consequently in vitro tissue quality [1]. Unfortunately, little is known about the origin and type of these mechanical cues during in vivo condensations and how they should be mimicked.

In this work, the developing chick limb is studied as a model-system for mesenchymal condensation. The mechanical cues that influence the condensation are a direct result of the mechanisms that drive outgrowth the limb bud. Therefor a better understanding of limb bud outgrowth is needed. We propose an individual cell-based model where mesenchymal cells are represented as individual agents. Interactions between the cells are modeled using two types of forces. Passive attraction-repulsion forces are used to represent cell-cell adhesion and cortical tension. The effect of active cell protrusions is represented as a directed migratory force. The ectoderm surrounding the mesenchyme functions as a boundary condition, and is modeled as a visco-elastic material. Our simulations indicate that graded proliferation mediated by FGF and directed intercalations directed by a WNT3a gradient are needed to ensure limb bud elongation.

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Abstract **#2194**

GLOBAL AND REGIONAL ANALYSIS OF ECM MINERALIZATION AND TISSUE DENSITY-MODULUS CONVERSION FOR 3D BIOPRINTED BONE ORGANOIDS

Julia K. Griesbach[P][1], Jianhua Zhang[1], Caitlyn J. Collins[1], Marina Rubert[1], Ralph Müller[1]

[1] *Institute for Biomechanics, ETH Zürich, Zürich, Switzerland*

Corresponding author's email: julia.griesbach@hest.ethz.ch

Human-derived bone organoids are emerging alternatives to investigate disease treatments. However, non-destructive methods to monitor mechanical properties and extracellular matrix (ECM) within these organoids are lacking. ECM mineralization has been successfully used for tissue mineral density to modulus conversions in native bone. This study aims to create an automated approach to non-destructively assess ECM mineral density and corresponding mechanical properties of organoids from micro-computed tomography (micro-CT) images.3D-bioprinted graphene oxide-incorporated cell-laden scaffolds[1] were cultured over 56 days in compression bioreactors, monitored using weekly micro-CT and endpoint compressive stiffness. Images were divided into 27 subsets. Density histograms of each subset were fit with two Gaussian curves to differentiate between background and ECM (R2=0.996), enabling regional and global assessment of mineralization. Tissue density was converted to Young's modulus using several exponential functions for FE analysis.Mechanical loading (ML) resulted in higher weekly mineral density increases (9.8mgHA/week) and greater stiffness than static culture (CN) (5.8mgHA/week). The top third of the ML organoids showed significantly higher mineral density compared to the middle and bottom thirds. Regional mineral density from the bottom third correlated highly with organoid mineral density (0.92) and experimental stiffness (0.98). This allowed using representative subsets, reducing boundary condition errors and computing time. The function:E[kPa] = 62.53×10^6×ρ^7.4[gHA/cm3]+3resulted in a high correlation (R2=0.94) between FE- and experimental stiffness.In this study, we created an automated approach to non-destructively assess ECM density and corresponding mechanical properties of organoids. This will facilitate future research into organoid development, especially regarding mechanical properties and mechano-regulation.

Keywords

bone organoids; micro-CT imaging; FE analysis

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Abstract **#2195**

A HIGH-THROUGHPUT FUNCTIONAL SCREENING PLATFORM FOR DETERMINING THE EFFECTS OF GALACTIC COSMIC RADIATION ON HUMAN CARDIAC TISSUE

Devin B Mair[P1], Marcus AC Williams[1], Roald Teuben[1], Shunyao Lei[1], Nicholas Geisse[2], Kenneth Boheler[1], Leslie Tung[1], Deok-Ho Kim[1]

[1] *Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD*

[2] *Curi Bio, Inc, Seattle, WA*

Corresponding author's email: dmair1@jh.edu

Galactic cosmic radiation (GCR) contains high energy ions that pose significant health risks to humans. Exposure to GCR promotes degenerative tissue effects1, decreases cardiovascular function, and increases the risk of negative cardiovascular events in astronauts 2. With the increasing interest in interplanetary and long duration spaceflight missions, methods to mitigate GCR need to be developed. Despite decades of research, little is known about the effects of GCR at the cell and tissue scale due to use of other radiation species in previous studies. Recently, the NASA Space Radiation Laboratory (NSRL) has developed methods to simulate GCR in a more realistic manner3. However, dysfunction of cardiac contractility can be difficult to model in vitro. To enable assessment of the effects of GCR on cardiac tissue function, we designed, optimized, and validated a method to fabricate and simultaneously measure the contractility of 24 3D human engineered muscle tissues fabricated from induced pluripotent stem cell-derived cardiomyocytes. The substrate features an embedded magnet; as tissues contract, the magnet's displacement is quantitatively detected in a highly parallel manner using specialized sensors to measure contractile function and kinetics. Using this platform, we are exposing hundreds of these tissues to 3 different doses of simulated GCR at NSRL and, for the first time, elucidating its negative impacts on cardiac tissue function and integrity, cell viability, mitochondrial function, genetic stability, and proteostasis. Using this data, we are developing methods to mitigate these effects.

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Abstract **#2196**

A HUMAN IPSC-BASED 3D MICROPHYSIOLOGICAL SYSTEM FOR MODELING CARDIAC DYSFUNCTION IN MICROGRAVITY

Devin B Mair$_P$[1], Jonathan H Tsui[1], Ty Higashi[2], Marcus AC Williams[1], Eun Hyun Ahn[1], Peter H.U. Lee[3,4], Stefanie Countryman[5], Nathan J Sniadecki[2], Deok-Ho Kim[1]

[1] *Dept. of Biomedical Engineering, Johns Hopkins University, Baltimore, MD*

[2] *Dept. of Mechanical Engineering, University of Washington, Seattle, WA*

[3] *Department of Cardiothoracic Surgery, Southcoast Health, Fall River, MA 02720*

[4] *Department of Pathology and Laboratory Medicine, Brown University, Providence, RI 02912*

[5] *BioServe Space Technologies, Boulder, CO*

Corresponding author's email: dmair1@jh.edu

Spaceflight has a negative impact on the heart and the cardiovascular system1, but the cell and tissue scale effectors of this negative impact remain poorly understood. To address this, we developed a high-throughput microphysiological model of human cardiac tissue, derived from human induced pluripotent stem cells (hiPSCs). To generate engineered heart tissues (EHTs) that are physiologically-representative of human myocardium, we used a biocompatible and cardiac-specific decellularized extracellular matrix (dECM)-based electroconductive composite scaffold. These dECM hydrogels contain tissue-specific ECM proteins, obtained through decellularization, and when combined with reduced graphene oxide, they recapitulate native cardiac tissue-like stiffness and electroconductivity2. The EHTs were successfully launched to the International Space Station (ISS) where they remained for 28 days while cardiac contractile function was measured in real time using a magnetic force sensor system before being returned to Earth. Analysis of flight sample data revealed a degradation of contractile function in EHTs, including reduced twitch forces and an increase in arrhythmias. Transmission electron microscopy results indicate changes in sarcomeric structure, lipid accumulations, and mitochondrial defects in spaceflight samples. This correlates with previous studies showing that mitochondrial dysfunction acts as a central hub for spaceflight-induced damage3. Using a random positioning machine to simulate microgravity, we compare the effects of simulated microgravity to actual spaceflight. Finally, using simulated microgravity, we show the effects of various mitigation strategies, such as increased resistance to contraction and small molecule therapeutics.

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Abstract **#2197**

3D-PRINTED TITANIUM SCAFFOLDS NEGATIVELY AFFECT THE INTERPLAY BETWEEN MACROPHAGES AND SKELETAL PROGENITORS (IN VITRO)

Gabriele Nasello[P][1,2], Tim Herpelinck[1,3], Giulia Rossana Gulino[3], Liesbet Geris[1,2,3,4]

[1] *Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Belgium*

[2] *Biomechanics Section, Mechanical engineering department, KU Leuven, Belgium*

[3] *Skeletal Biology & Engineering Research Center, KU Leuven, Belgium*

[4] *Biomechanics research unit, GIGA in silico medicine, University of Liège, Belgium*

Corresponding author's email: gabriele.nasello@kuleuven.be

The regenerative process guided by bone scaffolds depends on how they alter the interaction between immune and tissue-specific cells, which defines their osteoimmunomodulatory properties [1]. Despite the growing interest on additive manufacturing for orthopedic regenerative medicine, osteoimmunomodulatory properties of 3D printed bone scaffolds have not been widely assessed. In this study, we investigated the effect of 3D printed titanium (Ti) scaffolds on macrophages and skeletal progenitor cells in vitro. Human monocytes were seeded on 3D printed Ti scaffolds and differentiated to unpolarized macrophages. Simultaneously, human periosteum-derived cells (hPDCs) were seeded in separate wells. After 5 days, condition media from macrophages was applied to hPDCs and vice versa. Osteoimmunomodulation was evaluated in terms of metabolite concentration in macrophage cell culture media and gene expression analysis of both cell types. Results showed that Ti scaffolds inhibit the reduction in glucose consumption and lactate secretion, associated to an anti-inflammatory state and induced by hPDC-derived conditioned medium. Moreover, Ti scaffolds also upregulated macrophage pro-inflammatory genes both in the presence and absence of hPDCs conditioning. Conversely, macrophages cultured without Ti scaffold upregulated pro-regenerative genes when conditioned with hPDCs-derived media. Importantly, hPDCs did not upregulate osteogenic genes when conditioned with media from macrophages seeded on Ti scaffolds. Overall, these data suggest that 3D printed Ti scaffolds might have a negative influence on the regenerative response, by inducing an inflammatory state that inhibits the immunomodulation from skeletal progenitors as well as osteogenesis. Research aiming at functionalizing Ti scaffolds should address both effects.

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Abstract **#2198**

SAFETY, EFFICACY, AND IMMUNOGENICITY ASSESSMENT OF HUMACYTE'S HUMAN ACELLULAR VESSEL FOR DIALYSIS VASCULAR ACCESS: LUNA SYSTEM

Jeffrey Lawson[1], Jakub Turek[2], Wojciech Witkiewicz[2], Maciej Zielinski[3], Rob Schutte, Justin Strader P[4]

[1] *Humacyte, Inc., Durham NC*

[2] *The Regional Specialist Hospital of Wroclaw, Poland*

[3] *Poznan University of Medical Sciences*

[4] *Humacyte, Inc.*

Corresponding author's email: Schutte@humacyte.com

This was a Phase 2, prospective, multicenter, open-label, single-arm study. The primary objective of this study is to evaluate the safety, efficacy and immunogenicity over 3 months after implantation of human acellular vessels (HAVs) manufactured using the commercial manufacturing system LUNA. At Month 3, primary patency and primary assisted patency were maintained in 29 of 30 subjects, and all subjects showed secondary graft patency. HAV infections and indications of mechanical failure or weakness of the HAV were not observed. The percentage of subjects who exhibited elevation from Baseline in Class I PRA levels at Month 2 (7%) is consistent with previous clinical studies of Aura- or Terra-produced HAVs in patients requiring hemodialysis (6%). Overall, the results of the LUNA-produced HAV to date do not indicate any particular safety or immunogenicity issues. A follow-up 12-month assessment is currently ongoing and results may show similar outcomes.

Abstract **#2199**

MESOPOROUS BIOACTIVE GLASSES DOPED WITH HYPOXIA-MIMICKING ELEMENTS

Szymon Salagierski[P][1], Kinga Halastra[1], Zuzanna Samol[1], Katarzyna Cholewa-Kowalska[1], Michał Dziadek[1,2]

[1] *Department of Glass Technology and Amorphous Coatings, AGH University of Science and Technology, Cracow, POLAND*

[2] *Faculty of Chemistry, Jagiellonian University, Cracow, POLAND*

Corresponding author's email: ssalagierski@gmail.com

Mesoporous bioactive glasses (MBGs) are characterised by outstanding surface area and high porosity. These textural properties lead to significantly enhanced bioactivity compared with other glasses. Furthermore, this provides the possibility to use MBGs as local drug delivery systems, which combined with the release of therapeutic ions (e.g. Cu2+ and Co2+) gives opportunity for the development of multifunctional biomaterials. Cu2+ and Co2+ ions released from bioactive glasses have been shown to induce formation of new blood vessels by mimicking hypoxia (low oxygen concentration).

The aim of this work was to explore bioactivity and dissolution profiles of hypoxia-mimicking elements (Cu2+ and Co2+ ions) from MBGs and also to investigate the effect of these ions on the textural properties of MBGs. The Cu- and Co-doped MBGs with composition of 80SiO2–16CaO–4P2O5 (mol%) were produced using sol-gel technique coupled with evaporation-induced self-assembly process. CuO and CoO was substituted for CaO in the amounts of 1-5 mol%. MBGs were analysed using TEM, SAXS, and BET. MBGs were incubated in simulated body fluid (SBF) for 14 days. Changes in ion concentration in SBF were monitored using ICP-OES. FTIR and SEM/EDX were used to evaluate structural, morphological, and chemical changes of MBGs during incubation in SBF.

The results indicated that Cu2+ and Co2+ ions affect bioactivity and dissolution of MBGs to a great extent. Furthermore, the presence of hypoxia-mimicking elements altered their textural properties (surface area, porosity, pore size and structure).

This work was supported by the National Science Centre, Poland Grant no. 2019/32/C/ST5/00386 (MD).

Keywords

mesoporous bioactive glasses; therapeutic ions; textural properties

Abstract **#2200**

INFLUENCE OF VARIOUS STERILIZATION TECHNIQUES ON THE PROPERTIES OF MATERIALS ENRICHED WITH POLYPHENOLIC COMPOUNDS EXTRACTED FROM SALVIA OFFICINALIS L.

Szymon Salagierski[P][1], Kamila Checinska[1], Michal Dziadek[1,2], Kinga Dziadek[3], Barbara Zagrajczuk[1], Aneta Kopec[3], Katarzyna Cholewa-Kowalska[1]

[1] *Department of Glass Technology and Amorphous Coatings, AGH University of Science and Technology, Cracow, POLAND*

[2] *Faculty of Chemistry, Jagiellonian University, Cracow, POLAND*

[3] *Department of Human Nutrition, University of Agriculture in Krakow, Cracow, POLAND*

Corresponding author's email: ssalagierski@gmail.com

Due to the dynamic development of regenerative medicine, materials for bone tissue regeneration should exhibit bioactive, anti-inflammatory, antibacterial, antioxidant and anticancer properties. Extending the functionality of biomaterials is possible thanks to the enrichment of biomaterials with drugs or active substances. However, due to the contact of biomaterials with the patient's body, the materials are subjected to various sterilization techniques. Therefore, the aim of the research was to investigate the influence of various sterilization techniques on the properties of biomaterials.

The solvent casting method was used to produce polymer films (PCL) and composites modified with bioactive glass (BG). The materials were modified with polyphenolic compounds (PPh) extracted from sage (4.5 wt.%). Melt-derived and sol-gel-derived BG particles (d50 - 2 µm) with the composition (mol%) 40SiO2-54CaO - 6P2O5 (30% by weight) were used as the modifying phase. After ethylene oxide, gas plasma sterilization and UV-C sterilization, wettability and surface energy, the in vitro release of polyphenols and the antioxidant activity (ABTS) of the film were measured.

The influence of sterilization processes on the tested films turned out to be varied and dependent on the composition of materials. Moreover, for each of the materials, different sterilization methods showed different effects on the properties of the materials. Therefore, for the materials we test, the target type of sterilization should be selected individually. The possibility of changing the parameters of individual sterilization processes may constitute an important direction for further research.

This work was supported by the National Science Centre, Poland Grant no. 2017/27/B/ST8/00195 (KCK).

Keywords

biomaterials; sterilization; polyphenols

Abstract **#2201**

EXTREMELY LOW FREQUENCY ELECTROMAGNETIC STIMULATION AS A TREATMENT FOR ISCHEMIC STROKE: INVOLVEMENT OF NITRIC OXIDE

Annelies Bronckaers[P][1], Hannelore Kemps[1], Lena Perez[2], Lotte Alders[1], Jana Van Broeckhoven[1], Sebastien Foulquier[3], Sarah Lambrechts[3], Chantal Dessy[4], Laurent Dumas[4], Pierre Sonveaux[4], Robin Lemmens[5], Bert Brone[1]

[1] *Hasselt University, Uhasselt, Biomedical Research Institute (BIOMED), Agoralaan Building C, 3590, Diepenbeek, Belgium*

[2] *Universidad de Oriente, Centro Nacional de Electromagnetismo Aplicado (CNEA), Ave de las Américas s/n, 90900, Santiago de Cuba, Cuba*

[3] *Department of Pharmacology and Toxicology, School for Mental Health and Neuroscience, Maastricht University Medical Center+, Universiteitssingel 50, 6229 ER, Maastricht, The Netherlands*

[4] *Pole of Pharmacology and Therapeutics, Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain (UCLouvain), Avenue Hippocrate 57 box B1.57.04, 1200, Brussels, Belgium.*

[5] *VIB, Center for Brain & Disease Research, Laboratory of Neurobiology, Herestraat 49, 3000, Leuven, Belgium*

Corresponding author's email: annelies.bronckaers@uhasselt.be

Despite the high prevalence and devastating outcome, only a few treatment options for cerebral ischemic stroke exist. Previously, our group demonstrated that 'extremely low frequency electromagnetic stimulation' (ELF-EMS)' increased survival, reduced infarct size and improved neurological outcome in a rat model of global ischemia. The beneficial effects of ELF-EMS were inhibited by a general nitric oxide synthase inhibitor L-NMMA, indicating that nitric oxide (NO) is key in the actions of ELF-MF1. Since NO is a known vasodilator, we examined whether ELF-EMS was able to stimulate cerebral blood flow and investigated the activated intracellular pathways.

We demonstrated that ELF-EMS increased NO production in a human microvascular endothelial cells (HMEC-1), which was accompanied by enhanced phosphorylation of Akt and endothelial NOS (eNOS). Laser Speckle Contrast imaging (LSCI) proved that ELF-EMS increased the perfusion of the cerebral vasculature in both control and ischemic stroke mice. Moreover, application of ELF-EMS reduced lesion volume following ischemic stroke in collateral-rich C57BL/6 mice, but not in collateral-scarce BALB/c mice. These findings suggest that decreased lesion sizes in response to ELF-EMS results from improved cerebral collateral blood flow. Furthermore, ELF-EMS induced angiogenesis in the chicken chorioallantoic membrane assay and it enhanced key steps of the angiogenic cascade in vitro (endothelial cell proliferation, migration and tube formation).

In conclusion, we showed that ELF-EMS is a promising strategy to treat ischemic stroke by enhancing cerebral perfusion. In addition, because of its potential to induce angiogenesis it can also be an attractive strategy for other tissue regeneration purposes.

Keywords

Ischemic stroke; Angiogenesis; vasodilation

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Abstract **#2202**

DEFINING THE CONTRIBUTIONS OF THE MATRISOME TO THE PHYSICAL PROPERTIES OF THE BOVINE OVARY TO SUPPORT THE DESIGN OF AN ENGINEERED OVARIAN ENVIRONMENT.

Nathaniel Henning$_P$[1,2], Grace Schwartz[1,2], Monica Laronda[2,1]

[1] *Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL*

[2] *Department of Endocrinology, Department of Surgery, and Stanley Manne Children's Research Institute Ann & Robert H. Lurie Children's Hospital of Chicago*

Corresponding author's email: nathaniel.henning@northwestern.edu

Chemotherapy and radiation used for cancer and other treatment can cause depletion of the ovarian reserve, or primordial follicles, resulting in premature ovarian insufficiency (POI). Our goal is understanding the physical and biochemical properties of the ovarian microenvironment and their roles in folliculogenesis to inform a bioprosthetic ovary design that restores fertility and hormone function.

We mapped the rigidity (via Young's Modulus) of the ovary using atomic force microscopy (AFM) finding a significant decrease in rigidity from the cortex (8.87±0.80 kPa) to the medulla compartments (1.04±0.14 kPa). In order to associate these differences with specific ovarian features, we performed higher resolution (100µm) mapping across 3 zones (cortex, intermediate and deep medulla) in a 25X10 grids. Data was superimposed over H&E stained tissue to correlate information with specific follicles, vessels and other features. We have further assessed the specific contribution of matrisome proteins to these physical properties using compartment-derived decellularized ECM hydrogels. Additional investigation on how proteins of interest, such as EMILIN1 and AGRN, influence primordial follicle activation is ongoing using specifically designed hydrogels.

This work defines the physical properties of the ovarian microenvironment and develops tools to further investigate the role of specific proteins on folliculogenesis. This feeds our underlying knowledge towards creating an affective bioprosthetic ovary.

Abstract **#2203**

CELL CHARACTERIZATION OF JUVENILE AND ADULT EQUINE SYNOVIAL MACROPHAGES

Susanne Roth$_P$[1], Antonia Troillet[1], Guilia Liso[2], Theresa Triebe[1], Walter Brehm[1]

[1] *Clinic for Horses, Faculty of Veterinary Medicine, University of Leipzig*

[2] *Saxonian Incubator for Clinical Translation, SIKT, University of Leipzig*

Corresponding author's email: susanne.roth@sikt.uni-leipzig.de

Macrophages play a pivotal role in the initiation and regulation of inflammatory processes in the joint. Hence it is suggested that they are involved in the initiation and development of degenerative joint diseases such as osteoarthritis. Pro- and anti-inflammatory macrophage subpopulations are already described humans. To promote the use of synovial explants from horses for further in-vitro studies and to describe the role of macrophages, species specific markers have to be established. Therefore, macrophages isolated from synovial tissue by digestion and migration were characterized. Further, immuno-characteristics of macrophages in paraffine embedded tissue samples were compared to frozen sections of synovial samples from premature, juvenile, affected and non-affected adult horses to develop a distinct marker panel for equine synovial macrophages.

Keywords

horse; synovium; macrophages

Abstract **#2204**

CURRENT ASPECTS OF TRANSLATIONAL CARTILAGE REPAIR

Henning Madry[P][1]

[1] *Universität des Saarlandes, Germany*

Corresponding author's email: henning.madry@uks.eu

Abstract **#2205**

STRUCTURED SOFT SOLIDS FOR TISSUE REGENERATION

Liam M Grover[P][1]

[1] *University of Birmingham*

Corresponding author's email: l.m.grover@bham.ac.uk

Soft solids have found a plethora of applications in biomaterials and tissue regeneration, due to their similarity in structure to the extracellular matrix and high water-content. A major factor that limits their practical use is their low viscosity prior to gelation and poor mechanical properties after gelling has occurred. Our research has focussed on ways that the structure of hydrogels can be modified, without significant chemical modification, to enable the production of materials that exhibit shear-thinning properties with rapid elastic recovery once shear has been removed. Such materials have a range of practical uses including: minimally invasive delivery by injection; spraying; and delivery to the ocular surface. In addition to this, the materials can be used as a supporting medium into which other materials can be extruded. This presentation will discuss how we have generated processes to manufacture such materials and how we have adapted them for use as supporting beds in additive layer manufacturing. We will discuss how we have used this approach to manufacture and maintain complex cultures containing multiple cell types as analogues of cartilage, skin and bone

Abstract **#2206**

ROLE OF PERIOSTEUM AND SKELETAL MUSCLE MESENCHYMAL PROGENITORS IN BONE REGENERATION

CELINE COLNOT$_P$[1], Anais Julien[1], Ester Martinez-Sarra[1], Simon Perrin[1], Marine Luka[2], Michael Menager[2], Frédéric Relaix[1]

[1] *INSERM U955, UNIV PARIS EST CRETEIL*

2 INSERM U1163, Imagine Institute

Corresponding author's email: celine.colnot@inserm.fr

Bone exhibit great capacities to regenerate due to tissue-specific stem cells, i.e. skeletal stem/progenitor cells from periosteum and bone marrow. However, bone fails to heal properly in 10% of bone injuries and delayed healing is increased to 40% in patients with soft tissue damage associated with bone fracture. The role of skeletal muscle in bone repair is well recognized clinically but the underlying cellular and molecular mechanisms are poorly understood.

We show that mesenchymal progenitors residing in skeletal muscle adjacent to bone also participate in cartilage and bone formation during bone regeneration. We used single cell RNAseq analyses to characterize the periosteum and skeletal muscle mesenchymal progenitors, their response to bone injury and the impact of musculoskeletal trauma on the cellular response to injury. The results elucidate the diversity of skeletal stem/progenitors during bone repair and the role of skeletal muscle mesenchymal progenitors as a source of persistent fibrosis in musculoskeletal trauma. The findings suggest that new pharmacological and cell-based approaches can be developed to improve musculoskeletal regeneration by targeting skeletal muscle adjacent to bone.

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Abstract **#2207**

ASSEMBLY OF MOLECULAR AND COLLOIDAL BUILDING BLOCK TO GROW STRUCTURED TISSUES

Laura De Laporte$_P$[1,2,3]

[1] *DWI-Leibniz Institute for Interactive Materials*

2 RWTH University Aachen - ITMC

3 University Hospital RWTH Aachen - AME (AMB)

Corresponding author's email: delaporte@dwi.rwth-aachen.de

We apply polymeric molecular and nano- to micron-scale building blocks to assemble soft 3D biomaterials with anisotropic and dynamic properties. Microgels and fibers are produced by technologies based on fiber spinning, microfluidics, and in-mold polymerization. To arrange the building blocks in a spatially controlled manner, self-assembly mechanisms and alignemnt by external magnetic fields are employed. Reactive rod-shaped microgels interlink and form macroporous constructs supporting 3D cell growth. On the other hand, the Anisogel technology offers a solution to regenerate sensitive tissues with an oriented architecture, which requires a low invasive therapy. It can be injected as a liquid and structured in situ in a controlled manner with defined biochemical, mechanical, and structural parameters. Magnetoceptive, anisometric microgels or short fibers are incorporated to create a unidirectional structure. Cells and nerves grow in a linear manner and the fibronectin produced by fibroblasts is aligned. Regenerated nerves are functional with spontaneous activity and electrical signals propagating along the anisotropy axis of the material. Another developed platform is a thermoresponsive hydrogel system, encapsulated with plasmonic gold-nanorods, which actuates by oscillating light. This system elucidates how rapid hydrogel beating leads to a reduction in cell migration, while enhancing focal adhesions, native production of extracellular matrix, and nuclear translocation of mechanosensitive proteins, depending on the amplitude and frequency of actuation.

Keywords

microgel assembly; spinal cord repair; Anisogel

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Abstract **#2208**

A CLINICIAN'S PERSPECTIVE ON ADDITIVE MANUFACTURING IN OTOLOGY

Aaron Remenschneider$_P$[1]

[1] *Massachusetts Eye and Ear Infirmary*

Corresponding author's email: AARON_REMENSCHNEIDER@MEEI.HARVARD.EDU

The care of patients with otologic disorders is challenging given the intricate anatomy of the ear and the fragile nature of the auditory pathway. Patient outcomes following middle and inner ear procedures could stand to benefit from novel approaches to tissue, bone and cellular implants. The field of additive manufacturing has made significant recent progress in refining the resolution and biocompatibility of implantable materials, many of which are applicable to otologic applications. Furthermore, the ability to customize patient specific implants permits a tailored, personalized approach to patient care. This presentation will provide a state-of-the-art review of how additive manufacturing will impact the management of otologic disease. Specifically, we will discuss how 3D printed implant form affects is post-implant function. This talk will also explore the impact of advances in additive manufacturing on preoperative surgical planning, surgical simulation, biomimetic and patient specific implants and drug delivery in the treatment of ear disorders. We will explore 21st century approaches to solve persistent problems in otologic patients with a focus on late-stage translational science.

Abstract **#2209**

LOCAL AND SYSTEMIC DELIVERY OF FUNCTIONALIZED PLATINUM-BASED DRUGS FOR TREATMENT OF BONE METASTASES

Sander Leeuwenburgh[P][1,2], Michele Iafisco[2], Nicola Margiotta[3], Alessandra Barbanente[3], Robin Nadar[1]

[1] *Radboud University Medical Center, Nijmegen, The Netherlands*

2 Institute of Science and Technology for Ceramics (ISTEC), Faenza, Italy

3 Università degli Studi di Bari Aldo Moro

Corresponding author's email: sander.leeuwenburgh@radboudumc.nl

Current options for treatment of bone metastases are only temporarily effective because metastatic disease is ultimately an incurable conditions. Consequently, current treatments are only palliative. Therefore, new modalities for the treatment of bone metastases are urgently required. This presentation focuses on treatment of bone metastases by exploiting the potential of systemically or locally delivered Pt-based anticancer drugs. With respect to systemic delivery of these anticancer drugs, a novel radioactive bisphosphonate-functionalized platinum-based drug (195mPt-BP) was successfully synthesized to combine the diagnostic, radio- and chemotherapeutic properties of 195mPt with the bone-targeting characteristics of bisphosphonates. We confirmed for the first time that targeted delivery of these bone tumor-seeking radioisotopes induces killing of cancer cells in bone tumors due to the effects of Auger therapy. In the second part of this presentation, novel local delivery strategies are presented to deliver Pt-based anticancer drugs from hydroxyapatite nanoparticles into bone defects created by resection of bone tumors. These platinum-functionalized hydroxyapatite nanoparticles effectively killed breast cancer cells in zebrafish models. Furthermore, local co-delivery of platinum and selenium anticancer drugs from hydroxyapatite nanoparticles facilitated effective killing of cancer cells without harming co-cultured stem cells. Overall, this presentation shows how bisphosphonate-functionalized Pt-based anticancer drugs can be used to eradicate cancer cells in metastatic bone, thereby opening new avenues of research on treatment of metastatic bone disease.

Keywords

bone cancer; targeted delivery; drug delivery

Abstract **#2210**

BIOINSPIRED HYDROGELS FOR REGENERATIVE MEDICINE: TWO APPLICATIONS

Christophe Marquette[P][1]

[1] *3d.FAB - Université Lyon1*

Corresponding author's email: christophe.marquette@univ-lyon1.fr

Producing in vitro large pieces of tissue or organ is already within reach of few bioprinting laboratories. Advances in 3D printing, 3D bioprinting, but also complex tissue engineering and 3D tissue maturation are some of the key technologies that will make this happen.

Nevertheless, nothing will happen without informed strategies for biomaterial formulation. Here we will present a bioinspired approach for hydrogel formulations (1, 2) and their use as bioinks for regenerative medicine. In this approach, the lesson learned from the human tissue properties such as water content, biomolecules and mechanical properties, were used to design and validate proliferative and regenerative bioinks.

Two example of translational applications of the bioinspired hydrogel will be use to illustrate the approach: breast cancer reconstruction and skin regeneration (3).

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Abstract **#2211**

DEVELOPMENT OF A PEO-PROCESS TO GENERATE A MICRO-POROUS SURFACE STRUCTURE ON TI6AL4V-IMPLANTS

Thomas Derra$_P$[1]

[1] *Meotec GmbH*

Corresponding author's email: thomas.derra@meotec.eu

During the last decades, the development of orthopaedic implants has made great progress in adjusting the used materials to individual defect-related requirements. In the case of metallic implants new materials are still tested and alloys are optimized regarding biocompatibility and mechanical properties. In addition to shape and material of implants, the surface conditions have recently moved into focus of research. Interactions between implant surface and surrounding tissue can be influenced concerning osseointegration and soft tissue reactions. Furthermore, bioactive modifications can support bone growth and reduce risks of autoimmune and inflammatory diseases.

A promising surface modification for Ti6Al4V-implants is the Plasma Electrolytic Oxidation (PEO). PEO is an electrochemical technology that induces the transformation of a metal surface into a porous oxide ceramics surface. The resulting surface layer can be adjusted in thickness, porosity and chemical composition.

Meotec, as partner of the EVPRO-consortium, aims to use the PEO-technology to produce an implant surface on Ti6Al4V that supports the incorporation of MSC-EV loaded hydrogels into revision endoprosthesis. To reach better incorporation properties, the porosity and stability of the surface layer are focused, while the biocompatibility must be maintained. Only selected electrolytes are candidates for the PEO-process since their chemical elements migrate into the surface layer and support the resulting titanium oxide ceramics. Meotec conducted parameter studies with different electrolytes and found a process window for an electrolyte containing NH4H2PO4 and H2SO4. The resulting surface layer has a sufficient porosity and stability. Further tests will show the reproducibility and the interaction with hydrogel.

Abstract **#2212**

INJECTABLE AND 3D PRINTABLE GELATIN HYDROGELS RELEASING NITRIC OXIDE OR HYDROGEN PEROXIDE FOR TISSUE REGENERATION

Ki Dong Park$_P$[1]

[1] *Ajou University*

Corresponding author's email: kdp@ajou.ac.kr

Injectable hydrogels have been widely used as bioactive materials for tissue regeneration, owing to their extracellular matrix mimicking properties and minimally invasive surgical procedures. However, the inadequate vascularization of 3D injectable constructs limits their success after implantation. Currently, hydrogen peroxide (H2O2) nitric oxide (NO) are reactive molecules that are involved in many physiological processes such as immune responses, apoptosis, inflammation, etc. In particular, the angiogenic activities of H2O2 and NO were widely applied in the wound healing process and tissue repair/regeneration. In our lab, we developed injectable gelatin hydrogels that can control H2O2 / NO release to induce angiogenesis. In our approach, phenol moieties were conjugated onto the gelatin backbone to enable crosslinking of them for hydrogel formation via enzymatic crosslinking reaction of horseradish peroxidase (HRP). The gelation time, mechanical properties, and degradation of hydrogels were easily controlled by varying concentrations of HRP and H2O2. In vitro release studies demonstrated ed that the release behavior of H2O2 and NO from the hydrogel matrices can be precisely controlled by varying the concentrations of encapsulated glucose oxidase and copper ions, respectively. The hydrogels with optimal conditions enhanced the cellular activities of endothelial cells (proliferation, migration, and tube formation) in vitro and facilitated neovascularization in ovo. Our results suggest that H2O2/NO-releasing gelatin hydrogels can be utilized as advanced materials for tissue regenerative medicine and 3D bioprinting applications.

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Abstract **#2213**

CONTROL OF THE ADAPTIVE IMMUNITY BY LYMPH NODE STROMAL CELLS

Reina Mebius[P][1]

[1] *Amsterdam UMC location VUmc*

Corresponding author's email: r.mebius@amsterdamumc.nl

Lymph nodes are strategically located throughout the body in order to drain the tissues. They greatly enhance the odds that rare antigen-specific T cells encounter dendritic cells that present the antigen derived from the tissues. Stromal cells are crucially involved in the formation of lymph nodes through interacting with hematopoietic lymphoid tissue inducer cells (LTi cells). Once these structures are formed, lymph node stromal cells differentiate into the different stromal cell subsets, which are critical for organizing specialized microenvironments within lymph nodes, providing survival signals, guidance to immune cells leading to efficient cellular interactions.

One additional function of lymph node stromal cell is their capacity to present antigens, by which they shape immunity through inducing MHC-I-dependent deletion of self-reactive CD8+ T cells and MHC-II-dependent anergy of CD4+ T cells. We have shown that MHC-II expression by lymph node stromal cells is additionally required for homeostatic maintenance of regulatory T (TREG) cells and that in the absence of MHC-II expression on lymph node stromal cells, CD4+ as well as CD8+ T cells become activated. We now show that presentation of self-antigen in MHC-II by lymph node stromal cells resulted in the conversion of naive CD4+ T cells towards TREG cells.

In order to allow the study of human lymph node functioning we are currently creating a 3D organotypic in vitro model containing at first lymph node stromal cells and antigen presenting cells.

Keywords

Lymph node; Immune tolerance; Auto immunity

Abstract **#2214**

CELL-BASED THERAPIES - A SET OF BOTH PROMISES AND CHALLENGES

Malgorzata Lewndowska-Szumiel[1,2]

[1] *Laboratory for Cell Research and Application, Medical University of Warsaw*

[2] *Department of Histology and Embryology, Medical University of Warsaw*

Corresponding author's email: malgorzata.lewandowska-szumiel@wum.edu.pl

Regardless of having several approved ATMPs, with a 30% representation of tissue engineered products, and about 5,000 registered clinical trials of cell-containing products (excluding T cells), our knowledge about the principles of cell-based therapies in regenerative medicine is still very limited. The fundamental questions still remains: Do cells survive after being delivered? For how long? Are the cells, with multipotency confirmed in vitro, still multipotent in host tissues? Which is the predominant therapeutic mode of action: delivery of components to restore tissue in situ or providing trophic and/or immunomodulatory agents? It is not surprising, as we look at it. Unlike traditional medicines, the seemingly identical ATMPs can vary radically in terms of cell number, detailed manner and number of applications, medium/scaffold used for cell delivery, details of in vitro manipulations prior to application - just to mention the most important variables that characterize each individual treatment.

At the same time cell-based therapies are extremely demanding in terms of scientific, technical, regulatory and financial challenges, which explains much pressure put on the breakthrough success and the establishment of new directions. A critical review of those issues, based on the available data and our own experience, shows that the last two decades have not been a waste of time, and the further directions toward development of effective cell-based therapies can be identified. Nevertheless, the current situation is like a race - shall we manage to implement effective tissue engineered products before their development is strangled due to over-regulation and dramatically high costs?

Abstract **#2215**

CELLULAR AND MOLECULAR FORCES AT THE MATERIAL INTERFACE

E. Ada Cavalcanti-Adam[P][1]

[1] *Max Planck Institute for Medical Research*

Corresponding author's email: eacavalcanti@mr.mpg.de

Mechanical and chemical properties of the extracellular environment regulate cell adhesion and migration. Designing materials and tools which allow the local control of cell-matrix and cell-cell adhesion is of great interest to address how specific receptor-ligand interactions affect cell mechanotransduction. In this work, we report on the development of surface functionalization strategies to control integrin clustering for the generation of molecular and cellular forces at the interface during adhesion. We will also present some examples on the crosstalk between integrins and growth factors in the regulation of cell adhesion and mechanotransduction signaling.

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Abstract **#2216**

INNOVATIONS TO MAXIMIZE THE GIFT OF ORGAN AND TISSUE DONATION

Prakash Rao$_P$[1], Dayanand Deo[1], Misty Marchioni[1]

[1] *Personalized Transplant Medicine Institute at NJ Sharing Network*

Corresponding author's email: prao@njsharingnetwork.org

Stem cells can be isolated from various human tissues including bone marrow (BM) and adipose tissue (AT). Our studies outline the processes to isolate adult stem cells from research-consented deceased donors. We have found that deceased donor stem cells are a viable alternative to living donor stem cells and may help meet the current demand for cellular products. As a transplant organization, our goal is to save and enhance lives. Our efforts have resulted in the creation of functioning cardiomyocytes, hepatocytes, chondrocytes, adipocytes, osteocytes, and islet cells from otherwise unusable donated tissue. Our results demonstrate the structural and functional integrity of these cells. These differentiated cell types may be used as bridges to transplant. These cells may be utilized in immunomodulation, cellular therapy, starting material for 3-D bioprinting, and organoids. The secreted proteins from these cells, secretome, may provide cues for cell attachment, migration, proliferation, and special organization, and me be involved in regulation of immune responses. We have further observed in a preclinical model of acute kidney injury (AKI), the administration of a mixture of stem cells and secretome appeared to reverse AKI. Maximizing the gift of donation is a priority for the Personalized Transplant Medicine Institute. Procurement and processing of stem cells from deceased donors could be a routine practice and will offer increased accessibility for patients awaiting stem cell therapies.

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Abstract **#2217**

ORGAN AND TISSUE MODELS BY DESIGN: A NEW FRONTIER FOR TISSUE ENGINEERING DEDICATED MATERIALS

Gianluca Ciardelli[1]

[1] *Politecnico di Torino*

Corresponding author's email: gianluca.ciardelli@polito.it

Despite its being around for more than 50 years, Tissue Engineering has brought little commercial translation, with only few products having achieved market success. However, the increased understanding of human biology and our capacity to replicate it through sophisticated technologies and tailor-made materials has stimulated optimistic market forecasts. The design of 3D replicas of living tissues is expected to gain increasing attention considering their potential in personalized medicine and in high-throughput testing of cosmetics, drugs and chemicals, where they contribute to reduce animal experiments. It is worth considering e.g. that ~75% of drugs approved for liver and cardiac pathologies are withdrawn from the market due to safety concerns which were not highlighted in pre-clinical testing. Moreover, bioengineered models represent a more realistic scenario in testing implantable devices (e.g., prostheses testing in bone models).Ad-hoc designed materials are expected to play a strategic role in this arena, since they recapitulate the multicomponent composition and complex hierarchical architecture of the native extracellular matrix (ECM). Despite this potential, new biomaterials are still under-exploited: for instance, polyesterurethanes are extremely promising as cardiac patches but their difficult pathway to FDA approval negatively affected their translation. The talk will focus on novel trends in materials design, functionalization, and processing into biomimetic structures for the development of experimental models that respond to current challenges in the testing and evaluation of chemicals, drugs, and implantable devices.This work has received funding from the European Union's Horizon 2020 research and innovation action under grant agreement No. 814495-EVPRO (www.evpro-implant.eu).

Keywords

tissue models; polymeric biomaterials; biomimetic

Abstract **#2218**

TRENDS IN MATERIALS-DRIVEN IN-SITU REGENERATION

Carlijn Bouten[P][1]

[1] *Eindhoven University of Technology*

Corresponding author's email: c.v.c.bouten@tue.nl

Material-driven in-situ regeneration is emerging as an approach to regenerate tissues or organs directly at their functional site inside the human body using cell-free scaffolds or hydrogels. The approach requires the design of intelligent, 'life-like' biomaterials and fundamental understanding of how such materials guide functional neo-tissue formation by interacting with the host's endogenous regenerative processes and immune responses under (patho)physiological conditions. Within the Materials-Driven-Regeneration (consortium, Dutch researchers from various disciplines have joined forces to address the research challenges of in-situ regeneration and educate the next generation of regmed scientists in this new area. Together, they address the dynamic reciprocity between materials and the biological processes of regeneration at the cellular, tissue and organ level; and investigate the roles of chemical, physical and bioactive modifications of materials for this purpose. Enabling Technologies, Clinical Translation and Talent platforms are established for acceleration, cross-fertilization and translation of research outcomes.

As an example of the program I will concentrate on in-situ tissue engineering of heart valves and vessels. Here, the challenge is to design scaffolds that function upon implantation and during the process of tissue formation and scaffold degradation; and provide the necessary cues for a stable and organized load-bearing extracellular matrix under harsh hemodynamic conditions. I will describe how biomimetic in-vitro models and computational analyses are used in direct comparison with in-vivo animal experiments to provide handles for the optimization of scaffold immunomodulatory and degradation properties, where immunomodulation is introduced via biophysical or bioactive properties of the scaffold.

Keywords

cardiovascular; in situ regeneration

Abstract **#2219**

CELL-ASSEMBLED EXTRACELLULAR MATRIX PRODUCED IN VITRO AS A TRUE BIOMATERIAL

Nicolas L'Heureux[P][1]

[1] *BIOTIS - University of Bordeaux / Inserm*

Corresponding author's email: nico4cells@gmail.com

Tissue engineering was, and still is, often simplified to the basic recipe: scaffold + cells = replacement tissue. In this equation, the scaffold is so often a synthetic polymer that the two concepts have become synonyms in the minds of many. Unfortunately, even the most inert synthetic polymers are recognized by the innate immune system as a foreign body to be destroyed. In many applications, this foreign body reaction will cause complications like obstructive fibrosis, thrombosis, and chronic inflammation. An alternative approach is to use biological materials to provide a scaffold that the body can recognize and work with. However, this means that the body's adaptive and innate immune system will also recognize and destroy xenogeneic proteins or extracellular matrix (ECM) proteins that have been denatured (i.e., damaged). This is why animal-derived implants are treated with powerful crosslinking agents, making them unrecognizable to the cells (and a sort of foreign material). This also explains why extracellular matrix proteins that have been chemically solubilized and re-assembled are rapidly degraded after implantation. Another alternative is to have human cells assemble an ECM in vitro that can be used as a strong, unprocessed, completely biological, human scaffold for tissue engineering and/or surgical repair. This talk will present how Cell-Assembled extracellular Matrix (CAM) can be produced as a sheet, as a thread, and as particles to provide a new toolbox to address various regenerative medicine challenges. Past, present, and future applications will be discussed with a special emphasis on the development of human textiles.

Abstract **#2220**

ADVANCED HUMAN STEM CELL-BASED MODELS FOR MIMICKING CARDIOVASCULAR DISEASE. TOWARDS PERSONALIZED MEDICINE

Robert Passier[P][1]

[1] *University of Twente*

Corresponding author's email: robert.passier@utwente.nl

Cardiovascular disease is the leading cause of death globally, representing approximately 30 percent of all death. Although in the last decades researchers have focused on the treatment of heart disease, there is currently no cure for this disease. In addition to genetic and environmental factors, which are considered to be key determinants of cardiac disease, impaired cardiac function may be exhibited by unexpected drug-induced side effects, called cardiotoxicity. Besides the safety concerns, unpredicted cardiotoxic side effects leading to late-stage drug attrition or withdrawal of drugs from the market is a financial catastrophe for the pharmaceutical industry. One major problem is the limited ability to accurately mimic human heart disease and to predict the effects of potential heart drugs on patients using the current in vitro assays and experimental animal models. Human pluripotent stem cell (hPSC)-derived cardiac cells can be used to set up human-relevant, patient-specific cell-based assays for biomedical and pharmacological research. Here, I will discuss the application of hPSC-derived cardiomyocytes to more accurately mimic human heart function in vitro and their potential for tissue engineering and regenerative medicine. Moreover, the use of patient-derived cells will allow personalized medicine (also called precision medicine), enabling accurate assessment of the disease phenotype and underlying mechanisms, drug discovery and drug toxicity testing, leading to better and safer drugs.

Keywords

3D tissue engineering; Cardia disease modelling; organ-on-chip

Abstract **#2221**

FRONTIERS IN CELL MODELLING

Manuela T. Raimondi[P][1]

[1] *Polytechnic University in Milano*

Corresponding author's email: manuela.raimondi@polimi.it

This talk provides the fundamental concepts behind the design of frontier cell models that I have invented in the last two decades. One concept is miniaturisation associated to full-thickness optical accessibility(1) to recreate and monitor in real time and in long term culture, the interstitial perfusion process of 3D natural tissues such as brain, bone marrow and lymph node tissues. Cell models based on this concept proved able to recapitulate in vitro several slowly-developing biological processes, such as the formation of a bone metastasis by breast cancer cells(2), the instruction of adaptive immune cells in a lymphnode(3) and the neuroprotective effect of mesenchymal stem cell secretome on pathological neurons. I have then connected these models to recapitulate mechanisms involving multiple body compartments, such as the microbiota-gut-brain axis in neurodegeneration(4) and the bone marrow-lymphnode axis in leukemic transformation. Another concept is miniaturisation of the 3D cell scaffold. In the aim to better control and monitor stem cell function, I miniaturised the culture substrates in my models by applying a microfabrication technique called two-photon laser polymerization(5). Using these 3D micro scaffolds, I was able to condition mesenchymal stem cells, neural precursor cells and embryonic stem cells towards maintenance of a greater stemness and multipotency/pluripotency, compared to conventional 3D scaffold culture. Finally, I have recently invented a revolutionary concept of implanting the cell model in a living organism to regenerate a micro vascular network anastomosed to the host, allowing for studies involving interactions of the cell model with the host immune system.

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Abstract **#2222**

A BIOMIMETIC APPROACH TO MECHANICAL LOADBEARING IN HYDROGELS FOR CARTILAGE IMPLANTS

Corrinus C van Donkelaar$_P$[1], Rienk H Schuiringa[1], Maria Pastrama[1], Keita Ito[1]

[1] *Eindhoven University of Technology*

Corresponding author's email: c.c.v.donkelaar@tue.nl

Hydrogels are commonly used in cartilage tissue engineering, as they provide suitable environments for chondrogenesis. However, hydrogels have the general *limitation* that they are mechanically inferior to natural cartilage, and cannot withstand the loads in a joint after implantation. We developed an approach to address this, in which the loadbearing functionality originates from osmotic pressure, generated by the swelling potential of a charged hydrogel that is restricted from swelling by a textile mesh. This is very similar to the mechanism by which natural cartilage physiologically functions. This presentation will demonstrate the performance of such a construct, with quantitative relations between swelling potential of the hydrogel and compressive stiffness of the final construct. For this purpose, hydrogels with a variation of fixed charges are used, which are swollen and mechanically restricted with or without the presence of a textile spacer fabric.

Further, initial results of cell cultures within these constructs are shown, to illustrate the potential of these load-bearing, reinforced hydrogels for creating cartilage implants.

Abstract **#2223**

STEM CELL ENCOUNTERS WITH STRESSFUL SITUATIONS: SURVIVAL OF THE FITTEST

Elaine Fuchs[P][1,2]

[1] *Howard Hughes Medical Institute*

[2] *The Rockefeller University*

Corresponding author's email: fuchs@rockefeller.edu

Barrier epithelial tissues such as the skin, lung and gut are the first line of defense between our body and the external environment. As such, their stem cells must take on multifaceted tasks, self-renewing, protecting themselves, rejuvenating and repairing their tissues and calling for help from the immune system when their barrier has been breached. These stem cells reside in protected niches and together they undergo complex crosstalk to coordinate the stem cells' behavior and tasks. When the tissue is challenged by environmental stresses, such as allergens, pathogens, wounding or oncogenic stress, the stem cells must be poised to cope. We use high throughput genetic and genomic approaches to learn at a molecular level how the stem cells' differentiation programs are primed to operate under environmental stresses and how stem cell interactions with their niches differ in homeostasis, wound repair and inflammation. Our global objective is to apply our knowledge of the basic science of epithelial stem cells to unfold new avenues for therapeutics.

Keywords

cancer; stem cells ; inflammation

Abstract **#2224**

BIOENGINEERING ORGANOID PATTERNING

Adrian Ranga[P][1]

[1] *KU Leuven*

Corresponding author's email: adrian.ranga@kuleuven.be

Organoids serve as increasingly widespread model systems for understanding the complex interplay between biochemical and physical signals which govern in vivo fate specification, patterning and morphogenesis. While some elements of these complex biological processes can now be recreated in vitro, the current reliance on reductionist culture conditions largely overlooks the extracellular factors which promote multicellular growth and reorganization, leading to stochastic and poorly controllable outcomes. We tackle the challenge of reconstructing the multifactorial in-vivo microenvironment by deploying synthetic matrices in combination with customized devices which allow us to spatiotemporally control and manipulate mechanical forces to study early development events, as well as to microvascularize these tissue constructs at increasingly large scale. Our work with organoid-based models of neural tube morphogenesis suggests that the way forward towards engineering reproducible and scalable patterned tissue constructs may involve harnessing cells' inherent capacities for self-organization by providing mechanically active and highly perfused microenvironments.

Keywords

organoids; bioengineering; mechanobiology

Abstract **#2225**

TAMING CELLULAR SELF-ORGANIZATION IN MICROENGINEERED SYSTEMS

Stefan Giselbrecht$_P$[1]

[1] *MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University*

Corresponding author's email: s.giselbrecht@maastrichtuniversity.nl

Stem cell-based organoids, mimicking the development, physiology or disease of various organs, have become an important tool in understanding fundamental biological mechanisms. However, tools facilitating a faithful recapitulation and precise manipulation of such multicellular systems are still lacking. More advanced in vitro systems embracing the geometric and dynamic complexity are required to gain an improved understanding of tissue patterning and separation, long-term maintenance of tissue architectures and organogenesis. Microfluidic systems, allowing for a controlled decoupling of biological compartments, will play a key role in decoding spatial and temporal characteristics of signaling cues and mechanisms of cell-cell and tissue-tissue interactions. To recapitulate morphogenetic processes in vitro, we are developing techniques to engineer spatiotemporally controlled environments to orchestrate self-organization of pluripotent stem cells. Here, we present tailored microengineered cell culture systems, e.g. for blastoids and gastrointestinal organoids, to study early embryonic development and organogenesis. The development of more sophisticated 3D cell culture systems will also be a vital step forward in addressing specific questions in the field of organoids related to maturation, reduction of off-target phenotypes and the development of higher-order tissue architectures in the future.

Abstract **#2226**

YOU ARE WHAT YOU EAT: MODULATING METABOLISM TO DIRECT STEM CELL FATE

Nick van Gastel[P][1]

[1] *De Duve Institute, UCLouvain, Brussels, Belgium*

Corresponding author's email: nick.vangastel@uclouvain.be

Unraveling the molecular regulation of stem cells is critical to understand the functioning of these cells in health and disease, and to leverage their potential for regenerative medicine. Once thought to be a mere consequence of the state of a cell, intermediary metabolism is now known to play a key role in dictating whether a stem cell proliferates, differentiates or remains quiescent. In this presentation, I will highlight the recent methodological advances that allow us to investigate stem cell metabolism. I will further discuss our findings on the role of cellular metabolism in skeletal stem cells during bone fracture healing, and the potential of targeting metabolic pathways in bone tissue engineering approaches.

Keywords

Stem cells; Cell metabolism

Abstract **#2227**

FLOW AND VASCULAR REMODELLING: INSIGHT FROM COMBINED TIME-LAPSE MICROSCOPY AND COMPUTATIONAL MODELLING

Elizabeth A.V. Jones[P1], Ashkan Tabibian[1], Siavash Ghaffari[2], Diego A Vargas[3], Hans Van Oosterwyck[3,4], Richard Leask[5]

[1] *Centre for Molecular and Vascular Biology, Department of Cardiovascular Sciences, KU Leuven, Belgium*

[2] *Keenan Research Centre for Biomedical Science, Saint Michael's Hospital, Toronto, Canada*

[3] *Biomechanics Section, Department of Mechanical Engineering, KU Leuven, Leuven, Belgium*

[4] *Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium*

[5] *Department of Chemical Engineering, McGill University, Montreal, Canada*

Corresponding author's email: liz.jones@kuleuven.be

Vessel adapt to flow through a process of vascular remodelling. We have limited understanding of the direct relationship between changes in flow and the consequence on the network morphology. Some techniques, such as ultrasound biomicroscopy and optical coherence tomography, can follow flow over time, but due to resolution limits, they can only assess changes to larger vessels. Our group has developed a time-lapse imaging technique that allows us to measure flow patterns with micron resolution and concurrently image the blood vessel morphology. We use developmental avian models as our vascular bed. We combine the flow imaging with computational fluid dynamics (CFD) to extract shear stress levels and other forces. We follow both flow and vascular morphology over a period of 10 to 12 hours. In this way, we can calculate the force that are present and image how the vasculature reacts in real time. We have also combined our time-lapse imaging technique with computational modelling to understand how these forces induce the observed changes. Our results have shown that sprouts form at shear stress minimum. Interstitial flow also plays a role, limiting sprout formation to the side of an avascular region that is at lower pressure. Lastly, we use all this insight to develop self-propelled particle (SPP) model to model how endothelial cells move in response to flow and use this to predict final vascular morphologies based on our measured flow patterns.

Keywords

Angiogenesis; Vascular remodelling ; Computational modelling

Abstract **#2228**

COMPUTATIONAL MODEL OF CELL FATE DECISION IN THE EARLY MOUSE EMBRYO

Geneviève Dupont$_P$[1]

[1] *Université Libre de Bruxelles*

Corresponding author's email: genevieve.dupont@ulb.be

Embryonic development is a self-organised process during which cells divide, interact and change fate according to a complex gene regulatory network. In the early mouse embryo, the lineage specification of inner cell mass (ICM) cells into epiblast (Epi) and primitive endoderm (PrE) cells is tightly regulated by a gene regulatory network (GRN) and by extracellular signalling. We developed a realistic computational model of this GRN, which was shown to exhibit tri-stability and to account for the self-organized process of specification observed in vivo. The model also sheds light on various observations performed on wild-type or mutant embryos submitted to exogenous treatments that interfere with extracellular signalling. Models of increasing complexity, starting from the single cell level to a population of dividing cells interacting via extracellular signalling, are used to investigate the mechanistic origin of the experimentally observed spatial arrangement of the Epi/PrE cells in a salt-and-pepper pattern. The possible sources of noise, responsible for the initial symmetry breaking in murine early development, will also be discussed.

Abstract **#2229**

BIOENGINEERING CELLULAR MICRONICHES WITH PHOTOPOLYMERIZED HYDROGELS TO STUDY CURVATURE-INDUCED TISSUE REORGANIZATION

Marie Versaevel[P][1], Marine Luciano[1], Sylvain Gabriele[1]

[1] *University of Mons*

Corresponding author's email: marie.versaevel@umons.ac.be

Conventional 2D cell culture techniques have provided fundamental insights into key biochemical and biophysical mechanisms, however they do not fully capture the physical and chemical properties of the native cell microenvironment. Indeed, many tissues and organs present complex 3D environments with specific topographies, stiffness and biochemical patterns. These biochemical and mechanical properties of the cellular environment are related to the physiological function of the cells and tissues. The development of innovative cell culture substrates that reproduce the complexity of the 3D cell microenvironment is essential for physiologically-relevant in vitro studies and testing.

Here, we present an easy and robust method to produce hydrogel microniches in a wide range of stiffness, covering the whole physiological range (from 1 kPa to 250 kPa). We use the Irgacure 2959 photoinitiator to photopolymerize hydroxy-polyacrylamide (hydroxy-PAAm) hydrogels with ultraviolet illumination. By illuminating the hydrogel solution through an optical photomask with micrometer-scale patterns, we form 3D hydrogel microniches of varying geometries like corrugations, hemispheric and star-shaped bowls or villis.

In this work, these bioengineered microniches are used to study how matrix curvature impacts cell fate. By culturing epithelial tissues within bowl-shaped microniches of different diamaters and depth, we show that substrate curvature alters tissue morphology that results in nuclear deformation and orientation. This particular organization of cells within epithelial tissue generates numerous cellular exchanges between the niche and its surrounding environment. We envision that our 3D microniches will have potential applications in understanding how multicellular architectures organize and adapt to well-defined 3D structures.

Keywords

in vitro microenvironment; photopolymerization; topography

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Abstract **#2230**

TRANSCRIPTOMICS OF HEPATIC CELLS IN 3D ORGANOID SYSTEMS

Padma Rajagopalan[P][1]

[1] *Virginia Tech, Department of Chemical Engineering*

Corresponding author's email: padmar@vt.edu

Liver homeostasis requires the presence of both parenchymal and non-parenchymal cells (NPCs). However, systems biology studies of the liver have primarily focused on hepatocytes. Using an organotypic three-dimensional (3D) hepatic culture, we report the first transcriptomic study of liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) cultured with hepatocytes. Through computational pathway and interaction network analyses, we demonstrate that hepatocytes, LSECs and KCs have distinct expression profiles and functional characteristics. Our results show that LSECs in the presence of KCs exhibit decreased expression of focal adhesion kinase (FAK) signaling, a pathway linked to LSEC dedifferentiation. We report the novel result that peroxisome proliferator activated receptor alpha (PPARα) is transcribed in LSECs. The expression of downstream processes corroborates active PPARα signaling in LSECs. We uncover transcriptional evidence in LSECs for a feedback mechanism between PPARα and farnesoid X-activated receptor (FXR) that maintains bile acid homeostasis. We demonstrate that KCs in 3D liver models display expression patterns consistent with an anti-inflammatory phenotype when compared to monocultures. These results highlight the distinct roles of LSECs and KCs in maintaining liver function and emphasize the need for additional mechanistic studies of NPCs in addition to hepatocytes in liver-mimetic microenvironments.

Abstract **#2231**

RETINAL CELL THERAPY USING IPS CELLS

Masayo Takahashi[P][1,2]

[1] *Vision Care Inc.*

[2] *Kobe City Eye Hospital*

Corresponding author's email: takahashisec@vision-care.jp

Our aim is to develop outer retinal cell therapy using iPS cells. The first in man application of iPS cells started in 2013 for age-related macular degeneration. At first autologous iPS-derived retinal pigment epithelial (RPE) cell transplantation was done and then we proceeded to clinical research using HLA matched allogeneic iPSC-derived RPE cells from 2017. Immune responses to transplanted allogeneic cells could be controlled with topical steroids without systemic immunosuppressants. These clinical studies have confirmed the safety of iPS-derived RPE.

The next challenge is photoreceptor replacement. iPSC-retinal organoid transplantation is a promising treatment to restore visual function in degenerated retinas. We proved that (1) grafted immature retinal sheets maturated after transplantation in the eye, and (2) grafted photoreceptor cells could form synapses with host secondary neurons in adult mice with photoreceptor degeneration. (3) MEA (multi-electrode array) recordings showed that the grafted cells could elicit light responses in host ganglion cells. (4) The blinded mice could response to light stimuli in the behavior test after transplantation. With those findings as POC, we conducted a clinical study using retinal organoids for retinitis pigmentosa in two patients.

Based on these experiences, we are developing therapies for each case of outer retinal diseases. Our company regards regenerative medicine not only as the manufacture of final products but also as therapies. I will talk about the current status and future vision of retinal cell therapy.

Abstract **#2232**

SUPPORTING MODELLING IN TOXICOLOGY WITH NATURAL LANGUAGE PROCESSING

Marc Teunis[P][1], Marie Corradi[1]

[1] *HU - University of Applied Sciences*

Corresponding author's email: marc.teunis@hu.nl

Extracting information from text is a challenging task. Due to the increasing volumes of data, previously used paradigms such as systematic reviews are becoming impractical. The advancement of machine learning provides alternative solutions to this challenge. Qualitative and quantitative Information about effects of chemicals, chemical properties, molecular pathways, key-initiating events, in vitro assays, and dosage are contained within scientific literature, in-house testing reports and chemical and toxicological databases such as those of ECHA and ToxBank. To extract and organize relevant information from these sources, it seems valuable to employ deep learning Natural Language Processing techniques. This approach has already shown its value in some areas of toxicology, such as nanotoxicology and drug-induced liver injury. To support the data extraction for building toxicological models we propose the following approach. 1) Named Entity Recognition: We trained an NLP model using deep learning, that can recognize a number of entities relevant for the toxicological context. 2) Semantic relationships (linguistic dependencies) will allow to add qualitative (biological) and quantitative meaning to the entities. 3) Building semantic networks and relational graph visualizations will help provide context to chemical-organism interactions. 4) Connecting molecular ontologies and pathways aims to use existing ontologies to provide further insight in pathways and genes, related to effects of chemicals. With this data we can map underlying mechanisms that lead to toxicological effects and start building quantitative models for hazard and risk assessment.

This work is carried out within the ONTOX Horizon 2020 Project: https://ontox-project.eu/

Abstract **#2233**

WHY TERMIS

Jöns Hilborn[P][1]

[1] *Upsala University*

Corresponding author's email: hilborn@kemi.uu.se

Isn´t it exciting when you get your first proof-of-concept that your idea works? This is especially true when dealing with concepts that could help people with medical problems like lack of tissue or selecting the right combination of drugs for cancer treatment. When translating findings from bench to clinic, however, we reach the roadblocks of reality. We find that our source of scaffold materials varies between batches, our cells are too expensive to produce, our regulatory agency requests too much, and surgeons think that the procedure is complicated. Finally, the cost to bring our solution to the patient is too high for the small market we had in mind.

Could you have planned for this?

Much of the knowledge we are looking for we may find among colleagues in TERMIS. Before TERMIS was founded in the year of 2005 there were (competing) societies scattered over the world. To benefit from global knowledge, the idea was born to create ONE Society providing the best meeting in the field. We now have meetings that gather all competencies for our planning. Many of us, also would like to advance beyond the state-of-the-art and we are exploring new technologies, like 3D printing, IPSC, and extracellular vesicles with the hope that it will provide a breakthrough – but will it?

Here I would like to cite a Danish proverb: “It is very hard to predict, especially the future.”

Abstract **#2234**

HYALURONIC ACID BASED HYDROGELS FOR ANTIBODY DELIVERY AND 3D-BIOPRINTING

Tina Vermonden[P][1], Blessing Ilochonwu[1], Carl Schuurmans[1], Wim Hennink[1], Yu Shrike Zhang[2]

[1] *Utrecht University*

[2] *Harvard Medical School*

Corresponding author's email: t.vermonden@uu.nl

Hyaluronic acid (HA) is a highly negatively charged polysaccharide abundantly present in the body including in the vitreous of the eye. Injectable hydrogels based on hyaluronic acid therefore have high potential for delivery of therapeutic biomolecules for ocular diseases. Crosslinking HA by using dynamic bonds is an interesting approach to prepare these in situ gelling systems. On the other hand, covalent crosslinking leads to mechanically stable materials suitable for preparation of scaffolds by 3D printing.

Diels-Alder chemistry based hydrogels were prepared by mixing Furan-modified Hyaluronic acid (HAFU) with 4 arm-PEG10kDa-maleimide (4APM). The potential of this system for the treatment of ocular diseases was shown by testing the injectability into the vitreous body of an ex vivo porcine eye through a 29G needle. Importantly, 4APM-HAFU hydrogels were able to sustain delivery of bevacizumab for more than 90 days in vitro.

3D-printable hydrogels were prepared by photopolymerization of methacrylated hyaluronan. Complexing the negatively charged HA network with cationic polymers resulted in significant shrinking of the printed hydrogels due to electrostatic interactions. We showed that the use of opposite charges to shrink hydrogels is broadly applicable using different polycations. This shrinking effect was exploited to enhance the printing resolution.

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Abstract **#2235**

QUANTIFYING CELLULAR FORCES IN 3D: TOOLS AND APPLICATION TO ANGIOGENESIS

Hans Van Oosterwyck[P][1]

[1] *KU Leuven*

Corresponding author's email: hans.vanoosterwyck@kuleuven.be

Cellular forces play a crucial role in tissue and organ formation. In order for cells to organize into 3D multicellular entities they need to apply forces to neighbouring cells and their extracellular matrix. In addition, forces are modulators of molecular signaling, as they can influence molecular activity and interactions in a process that is known as mechanotransduction. As such, cellular forces are important for development, growth, regeneration and disease and assessing them can enhance our understanding of governing mechanisms, complementary to information stemming from cell and molecular biology assays.

In this talk I will give an overview of the tools we have developed in quantifying cellular forces in 3D in vitro systems. Having added the third dimension makes those tools more relevant for tissue engineering and regenerative medicine, but also comes with a price in terms of complexity. I will highlight some of the pitfalls related to the use of 3D Traction Force Microscopy (TFM), and the solutions we have developed in order to ensure a sufficiently accurate and efficient workflow (1). We have developed a stand-alone software called TFMLAB (2), that is freely available and that has the ambition to also be used by non-expert users, so that cellular force data becomes more accessible to different fields in life sciences. Finally, I will illustrate the applicability of these tools to the study of angiogenesis (3) and the role of cellular forces in microvascular disease.

Keywords

mechanobiology; traction force microscopy; angiogenesis

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Abstract **#2236**

4D PRINTING: A FOREFRONT MANUFACTURING APPROACH FOR SMART DEVICES.

Giovanni Vozzi[1], Irene Chiesa[1], Carmelo De Maria[1], Amedeo Franco Bonatti[1]

[1] *University of Pisa - Research Center "E. Piaggio" & Department of Information Engineering*

Corresponding author's email: giovanni.vozzi@unipi.it

In 2013, the term “four-dimensional (4D) printing” was introduced to denote the fabrication via additive manufacturing (AM) of structures capable of shape transform over time, under a predefined stimulus. Thus, 4D printed constructs are no longer static objects but programmable active structures that accomplish their function through a change, over time, in their physical and/or chemical properties when exposed to a predefined stimulus [1,2]. The key points of 4D printing are AM technologies, smart materials or combination of different materials, and external stimuli. Since 2013, the 4D printing approach has been in rapid expansion in several biomedical fields, including tissue engineering, and biomedical devices. Indeed, native tissues exhibit unique functions that are achieved through dynamic changes in tissue conformation. Thus, 4D printing may produce biologically active constructs that can alter their shapes upon desired stimulation, mimicking the native tissue movements, adapting to the 3D complex shapes of the human body, or providing appropriate stimuli to promote cell differentiation and activities [3]. Similarly, 4D printing has the potential to drive a significant transformation in the medical devices field, manufacturing customized implants able to grow up with the patient, devices that allow the use of minimally invasive surgical procedure, or perform their action without the need of electrical energy [4]. Here, we present the implementation of the 4D printing paradigm in practical applications of biomedical interest, such as bio-based medical devices for abdominal surgery and bioengineered platforms for neural tissue engineering, developed by the Biofabrication Group of the University of Pisa.

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Abstract **#2237**

CHAOTIC PRINTING OF LAYERED FILAMENTS WITH HIGH SURFACE AREA TO VOLUME RATIO FOR USE IN MESENCHYMAL STEM CELL PROLIFERATION

David Dean[P1,2], Ryan Hooper[3], Ryan Brune[4], Amanee Abu Arish[1,2], Raquel Tejeda[1,2,5], Ciro Rodriguez[5], Mario Alvarez[6], Grissel Trujillo[6]

[1] *Department of Materials Science and Engineering, The Ohio State University, Columbus, Ohio, USA*

[2] *Department of Plastic and Reconstructive Surgery, The Ohio State University, Columbus, Ohio, USA*

[3] *Department of Biomedical Engineering, The Ohio State University, Columbus, Ohio, USA*

[4] *Center for Design and Manufacturing Excellence, The Ohio State University, Columbus, Ohio, USA*

[5] *Department of Mechanical Engineering and Advanced Materials, Tecnológico de Monterrey, Monterrey, Mexico*

[6] *Department of Bioengineering, Tecnológico de Monterrey, Monterrey, Mexico*

Corresponding author's email: David.Dean@osumc.edu

Most current applications of biofabrication are for the production of devices that are aimed at modeling disease processes, screening drugs, or producing a device to be used in therapy, most often regenerative therapy. However, one of the most significant problems facing all cell-based therapies is the expansion of the relatively small numbers of donated by consented patients to the number of cells needed for therapeutic interventions. A single donor may contribute millions of cells. With or without the pooling of cells donated from multiple individuals, the therapeutic need for hundreds of millions to billions cells is a significant challenge. An example of the type of cell expansion that is needed for therapeutic interventions is the use of Bone Marrow-derived human Mesenchymal Stem Cells (BM-hMSCs) in leukemia therapies and, potentially, in regenerative medicine. Our group has recently demonstrated the ability to create layers within biofabricated filaments at nanometer to micrometer resolution with a novel technique, Chaotic Printing (CP). An ongoing study shows that CP offers promise as a technique for the proliferation of BM-hMSCs as it overcomes the limits on surface area to volume (SAV) seen in current cell expansion bioreactors. As SAV increases, a second challenge arises is the ability to provide sufficient nutrients and remove rapidly accumulating waste products. Our use of CP to intercalate fugitive and cell-bearing layers shows promise for this second challenge. Based on our recent results using this technology we anticipate that CP can be used to significantly improve BM-hMSC expansion over current bioreactor strategies.

Abstract **#2238**

USE OF ADVERSE OUTCOME PATHWAYS FOR THE IN VITRO PREDICTION OF LIVER TOXICITY

Mathieu Vinken[P][1]

[1] *Vrije Universiteit Brussel*

Corresponding author's email: mathieu.vinken@vub.be

Adverse outcome pathways (AOPs) are pragmatic tools in toxicology and human risk assessment with broad potential. AOPs are designed to provide a clear-cut mechanistic representation of toxicological effects that span over different layers of biological organization. Because of its unique location and function in the organism, the liver is a frequent target for systemic toxicity. Not surprisingly, several AOPs related to liver toxicity have been introduced over the fast few years. Focus will be put in this presentation on liver toxicity triggered by bile acid accumulation. This so-called cholestatic hepatotoxicity underlies many drug-induced liver injury cases and has also been associated with various other types of chemicals, including food additives, cosmetics and biocides. The present presentation will give an overview of the validation of this AOP on cholestatic liver injury. In particular, testing of the specificity and applicability domain will be presented along with its potential to predict cholestatic liver toxicity in vitro.

Abstract **#2239**

COLLABORATIVE ANALYSIS OF WHOLE-SLIDE TISSUE IMAGES WITH CYTOMINE OPEN-SOURCE RICH INTERNET APPLICATION

Raphaël Marée[P][1]

[1] *University of Liège*

Corresponding author's email: raphael.maree@uliege.Be

The Cytomine project (https://www.cytomine.org/) started in 2010 at University of Liège (https://uliege.cytomine.org) to build a rich web environment for the analysis of multi-gigapixel imaging data. This open-source software tool has been designed with the following objectives in mind: provide remote and collaborative principles, rely on data models that allow to easily organize and semantically annotate imaging datasets in a standardized way, efficiently support high-resolution multi-gigapixel images (e.g. whole-slide images in digital pathology), and provide mechanisms to readily proofread and share through the web image quantifications produced by machine/deep learning-based image recognition algorithms. By emphasizing collaborative principles, our aim with Cytomine is to accelerate scientific progress and to significantly promote image data and algorithm accessibility and reusability. Cytomine collaborative principles allow to break common practices where imaging datasets, quantification results, and associated knowledge are still often stored and analyzed within the restricted circle of a specific laboratory.

In this talk, we will summarize its main features [1] and applications in various domains, and give an overview of recent results and its extension to perform image analysis benchmarking (Rubens et al., 2020).

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Abstract **#2240**

LIGHT-ADDRESSABLE CELLULAR MATRICES WITH IN SITU SENSING AND ACTUATION FUNCTIONALITIES

Carmen Bartic[P][1]

[1] *KU Leuven*

Corresponding author's email: carmen.bartic@kuleuven.be

In order to decipher cellular functions, understand disease mechanisms and develop regenerative strategies, we need methods allowing modulating and detecting cellular activity with high spatio-temporal resolution, in 3D cell preparations. In this contribution, a multifunctional platform will be discussed where nanoparticle sensors and actuators are incorporated in a hydrogel supporting cell encapsulation. This system functions as a light-addressable cellular matrix, where 3D temperature gradients are produced locally and dynamically using the localized surface plasmon resonance (LSPR) of gold nanorods (AuNRs) under near infrared (NIR) laser illumination. At the same time, the temperature changes are probed locally by monitoring the temperature induced changes on fluorescent nanothermometers. Plasmonic heating also deforms the hydrogel controllably and reversibly, allowing to apply mechanical and thermal stimuli in a 3D cell preparation. The hydrogel deformation is quantified by means of inline holographic microscopy and in principle it can be to probe cellular mechanical properties. Combining the environment-sensitive and size-dependent optical properties of nanoparticles with soft hydrogels and light-patterning strategies allows tuning the properties of the cellular environment with sub-cellular resolution and can provide an efficient method for remote, highly localized cell stimulation with in situ parameter monitoring.

Keywords

active cell matrices; sensing; actuation

Abstract **#2241**

BIOPRINTING IN SPACE

Vladimir Mironov[P][1]

[1] 3D B

Corresponding author's email: vladimir.mironov54@gmail.com

Magnetic levitational bioassembly of three-dimensional (3D) tissue constructs represents a rapidly emerging scaffold- and label-free approach and alternative conceptual advance in tissue engineering. The magnetic bioassembler has been designed, developed, and certified for life space research. To the best of our knowledge, 3D tissue constructs have been biofabricated for the first time in space under microgravity from tissue spheroids consisting of human chondrocytes. Bioassembly and sequential tissue spheroid fusion presented a good agreement with developed predictive mathematical models and computer simulations. Tissue constructs demonstrated good viability and advanced stages of tissue spheroid fusion process. Thus, our data strongly suggest that scaffold-free formative biofabrication using magnetic fields is a feasible alternative to traditional scaffold-based approaches, hinting a new perspective avenue of research that could significantly advance tissue engineering. Magnetic levitational bioassembly in space can also advance space life science and space regenerative medicine.

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Abstract **#2242**

PATIENT-SPECIFIC MODELLING FOR OPTIMISING HEART VALVE IMPLANT DESIGN AND INTERVENTION

Sotirios Korossis[P][1,2]

[1] *Loughborough University*

[2] *Hannover Medical School*

Corresponding author's email: s.korossis@lboro.ac.uk

Understanding healthy and diseased tissue behaviour is of paramount importance in designing implants and surgical interventions for the clinical setting. However, the biomechanical and, for the case of cardiovascular tissues, haemodynamic behaviours of tissues are difficult to be assessed in vivo. In addition, tissue behaviour and function are specific to the individual patient and this needs to be accounted for in order to appropriately tailor patient-specific implants and/or interventions. In contrast to the practice of one-size-fits-all, tailoring the implant and/or intervention to the individual patient is considered to significantly improve implant survival and reduce risky and costly reoperations. Recent advancements in medical imaging offer important solutions for such patient-specific assessment of tissue behaviour and function. However, medical imaging is still limited to predominantly qualitative assessment. To this end, experimental and computational simulations have the potential to generate quantitative information of tissue behaviour and function, by complementing medical imaging and providing patient-specific assessments. This talk will present and discuss a number of experimental and patient-specific computational models that were developed at Loughborough University and Hannover Medical School with a view to assessing heart valve function in health and disease and optimise surgical intervention.

Keywords

Computational simulation; Heart valves; Experimental simulation

Abstract **#2243**

PLACENTAL CELL THERAPY FOR MUSCLE TRAUMA IN HIP FRACTURE PATIENTS: FROM PRECLINICAL TO CLINICAL PHASE III

Tobias Winkler$_P$[1]

[1] *Charité - Universitätsmedizin Berlin*

Corresponding author's email: tobias.winkler@charite.de

Mobility after hip fracture arthroplasty is critical but difficult to achieve. This can be related to the general condition of the patients and the additional muscle injury needed for joint exposition in these frail patients. Our studies have explored therapy with mesenchymal stromal cells (MSC) for skeletal muscle injuries and transferred this therapy from preclinical tests to clinics.

In a muscle injury model, we tested autologous and allogeneic MSC transplantation, using placenta-derived cells (PLX-PAD) for the latter. We translated this therapy by using iatrogenic muscle damage after total hip arthroplasty (THA) as a model system and conducted a prospective, randomized, double blind, placebo-controlled phase I/II study randomizing 20 THA patients (lateral approach) to transplantation with 300x106 (300M), 150x106 (150M) PLX-PAD or placebo into the injured gluteus medius muscles (GM).

Preclinical experiments showed improved functional muscle healing. Patients of the phase I/II study showed safety and an increase of GM strength and volume in the 150M group (p=0.0067) after 6 months accompanied by faster histological healing and a reduction of postoperative immunological stress.

Based on these results we conduct a phase III study (The HIPGEN study) treating hip fracture arthroplasty patients (N=240) with PLX-PAD (EU Horizon 2020 No 779293) currently enrolling in 20 international sites. Patients are followed for function, biomechanics, quality of life, muscle volume and immunological biomarkers.

Treatment with allogeneic cells could be a game changer not only in the treatment of hip fracture patients but also for other traumatic or iatrogenically induced muscle injuries.

Keywords

Hip Fracture; Cell Therapy; Muscle Trauma

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Abstract **#2244**

CLINICAL APPLICATION OF TISSUE ENGINEERED VASCULAR GRAFT IN CONGENITAL HEART SURGERY

Toshiharu Shinoka$_P$[1]

[1] *Nationwide Children's Hospital*

Corresponding author's email: toshiharu.shinoka@nationwidechildrens.org

In 2001, we developed the first tissue engineered vascular graft (TEVG) for use in congenital heart surgery and confirmed its significant potential via an initial clinical trial. Between September 2001 and December 2004, 25 patients underwent an extracardiac Fontan using tissue engineered vascular graft (TEVG) in Tokyo. There was no graft-related mortality occurred. There was no evidence of aneurysmal formation, graft rupture, graft infection, or calcification. Ten patients had asymptomatic graft stenosis. Six of 10 patients underwent successful balloon angioplasty. Angiographical assessment shows the growth of grafts. Based on our animal experimental data, we figured out that higher dose cell seeding on the graft could reduce the stenosis rate. We had applied new protocol to FDA for the second generation of TEVG, including a rapid cell seeding technique in 30 patients as Fontan conduits. The rational design of improved, second-generation TEVGs will be predicated on our understanding of the cellular and molecular mechanisms underlying the formation of TEVG stenosis. The feasibility of tissue engineering large caliber, autologous vascular conduits for use as venous interposition grafts for congenital heart surgery has been demonstrated in both large animal and in a human clinical trial. The carefully designed second clinical trial under the supervision of the FDA is underway in the United States.

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Abstract **#2245**

DEVELOPMENT OF CELL-ASSEMBLED 3D MAGNETICALLY RESPONSIVE TENDON CONSTRUCTS OFFERING THE PROSPECT OF EFFECTIVE TISSUE REGENERATION

Manuela E. Gomes$_P$[1]

[1] *3B's Research Group - I3Bs Research Institute-University of Minho*

Corresponding author's email: megomes@i3bs.uminho.pt

The limitations of currently used therapies together with the limited regenerative ability of tendons have motivated tissue engineering (TE) strategies to develop living tendon substitutes. However, the limited knowledge on tendon development and healing processes has hindered the design of TE procedures that more closely recapitulate tendon morphogenesis. Our lab has been exploring the development of unique cell-laden 3D magnetically responsive systems that recapitulate key features of the native tissue and that can be further remotely modulated both in vitro and in vivo by the application of external magnetic stimuli. We are exploring conventional and innovative tools such as multimaterial 3D bioprinting to design magnetic responsive systems mimicking specific aspects of tendon tissue architecture, composition and biomechanical properties, which, combined with adequate stem cells, shall render appropriate behavioural instructions to stimulate the regeneration of tendon tissue.

Despite the limited knowledge on tendon development and healing processes, it is known that inflammation plays a key role in tendon tissue healing. Persistently increased inflammatory cytokine levels have been associated to tendon degeneration and/or scar tissue formation upon injury and therefore may provide a target for assisting new therapeutic strategies. Therefore, we have been also focusing efforts on understanding possible uses of magnetic stimulus, in combination with magnetic responsive materials, to modulate inflammation in tendon/tendon cells, which can provide an additional valuable tool to trigger tissue regeneration instead of simple repair.

Acknowledgments: Hospital da Prelada for tissue samples; FCT project MagTT PTDC/CTM-CTM/29930/2017, ERC CoG MagTendon (772817) and Twinning Project Achilles (810850)

Abstract **#2246**

HUMAN PLURIPOTENT STEM CELL-DERIVED GASTROINTESTINAL ORGANOIDS AS NEW MODELS TO STUDY HUMAN ORGAN DEVELOPMENT, DIGESTIVE DISEASES AND DIABETES

James Wells$_P$[1]

[1] *Cincinnati Children't Research Foundation*

Corresponding author's email: james.wells@cchmc.org

Successful efforts to direct the differentiation of human embryonic and induced pluripotent stem cells (PSCs) into specific organ cell types in vitro have largely been guided by studies of embryonic organ development. Based on this, we can now direct the differentiation of human PSCs into gastrointestinal (GI) organoids including esophageal, gastric fundus, gastric antrum, small intestinal and colonic organoids. GI organoids contain complex epithelial structures and diverse cell types that are unique to their representative organ; esophageal organoids develop a stratified squamous epithelium, gastric organoids have a glandular epithelium that secrete digestive enzymes, and acid, and intestinal organoids secrete mucins and hormones and absorb nutrients. While the first generation of GI organoids were lacking important cell types and functions, we are now able to engineer additional complexity at will. For example we have engineered gastric, intestinal and colonic organoids to have functionally innervated smooth muscle generated colonic organoids with functional immune cells capable of triggering an inflammatory cascade in response to pathogenic bacteria. We have used PSC-derived organoids to identify the underlying mechanisms behind human GI diseases ranging from infections to malabsorption. We are further using organoids to uncover the molecular basis of congenital malformations including Hirschsprung's disease and esophageal atresia and to identify new pathologies in patients with complex GI diseases.

Abstract **#2247**

FABRICATION OF ENGINEERED VASCULATURE TO MODEL PATHOLOGICAL PROCESSES

John H Slater$_P$[1,2]

[1] *University of Delaware*

[2] *Department of Biomedical Engineering*

Corresponding author's email: jhslater@udel.edu

Many diseases either directly affect the cardiovascular system, hypertension and inflammation for example, or utilize the cardio and/or lymphatic vasculature during disease progression, cancer metastasis for example. While diseases that affect or utilize cardiovasculature can be studied in both human and animal models, high-resolution temporal imaging of cellular and subcellular events during these processes is often challenging due to the resolution limitations of existing in vivo imaging modalities. Accordingly, the ability to model these processes using engineered vasculature in fluidized, in vitro devices offers the opportunity to begin forming a better understanding of the cellular and molecular mechanisms occurring. Toward this goal, I will discuss various biofabrication techniques (sacrificial molding, laser-induced hydrogel degradation) to generate simplified, three-dimensional microvascular systems embedded in synthetic (poly(ethylene glycol)) or natural (collagen, fibrin) hydrogels. I will also discuss the implementation of these devices to form a better understanding of the cellular and molecular mechanisms that facilitate extravasation of circulating tumor cells during breast cancer metastasis and the potential role of pathological hemodynamics in dementia.

Abstract **#2248**

BIOHYBRID CARDIOVASCULAR IMPLANTS: A BRIDGE BETWEEN THE ARTIFICIAL AND THE NATURAL

Alicia Fernández Colino[P][1]

[1] *Department of Biohybrid & Medical Textiles (BioTex), AME-Institute of Applied Medical Engineering, Helmholtz Institute, RWTH Aachen University, Aachen, Germany*

Corresponding author's email: fernandez@ame.rwth-aachen.de

Biological systems in general, and native tissues in particular, display astonishing designs that have been defined and redefined by the process of evolution. While native organs are able to function in the majority of individuals through a lifetime, the reality is that current bioengineered cardiovascular substitutes are far from reaching the native performance. Therefore, the principles of bioinspiration and biomimicry should be embraced in order to develop functional implants. The transfer of the designs found in Nature to the lab represents a promising avenue to develop replacements able to cover the gap between the suboptimal performance of currently available replacements and patients in need of a life-saving implant. Here we propose the use of advanced materials together with innovative bioprocessing techniques to develop biohybrid implants that mimic the complexity of native tissues. The developed cardiovascular replacements combine the elasticity and hemocompatibility of novel genetically engineered polymers with the superior strength of fibrous components.

Abstract **#2249**

BOTTOM-UP ASSEMBLY FOR 3D TISSUE CONSTRUCTION

Shoji Takeuchi[P][1]

[1] *University of Tokyo*

Corresponding author's email: takeuchi@hybrid.t.u-tokyo.ac.jp

Rapid formation of three-dimensional, highly dense, and macroscopic tissues in vitro is attracting attention not only in regenerative medicine and drug discovery but also in a wide range of application fields such as food and robot engineering. Here, I will discuss several microfabrication techniques for the rapid construction of 3D tissue that can be used for cell therapy or cultured meat. We demonstrated a bottom-up tissue construction method using different types of cellular modules that serve as building blocks for thick and dense 3D tissues.

Abstract **#2250**

MAPPING AND PERTURBING CELL-INTRINSIC GENE AND PROTEIN REGULATORY NETWORKS IN EMBRYONAL CELLS: LESSONS FOR CELL-BASED TERM APPLICATIONS

Danny Huylebroeck$_P$[1,2]

[1] *Dept. Cell Biology, Erasmus University Medical Center (Rotterdam, NL)*

[2] *Dept. Development and Regeneration KU Leuven (Leuven, BE))*

Corresponding author's email: d.huylebroeck@erasmusmc.nl

Developmental signaling is repeated during tissue/organ regeneration and used to expand cells with regenerative potential. The multipotent BMP-system has many components, which are studied in lab animals and cell culture, often by loss-of-function or other perturbations. Such studies encompass single intracellular signaling effectors and transcription factors determining cell fate, maturation and behavior. This large set of BMP components is exquisitely connected via autoregulation, synexpression and feedback control at the transcriptional level of all component genes. Hence, modulation of one component has consequences for other ones in cell transitions. We have started to map such consequences, including within single cells and at various time points and cell states, using BMP component perturbations (n=96; esiRNA, CRISPR/Cas), and combine profiling of temporal transcriptional changes, single-cell heterogeneity, and gene-to-gene interactions. One lesson is that 85% of significant gene-to-gene interactions are restricted to one specific cell transition or stage/state, are driven by few transcription factors and, within such system, there exist low vs. high perturbation-sensitive genes. Therefore, interpretation of the consequences of single-gene perturbation in cell lineage progression should occur with caution and consider cell stage and transition. Our results also have consequences for many of the CRISPR-KO based proposed improvements of tailored stem cells.

Abstract **#2251**

Lorenzo Piemonti[P][1,2]
[1] *Diabetes Research Institute, IRCCS OSpedale San Raffaele, Milan, Italy*
[2] *Vita-Salute San Raffaele University, Milan, Italy*

Corresponding author's email: piemonti.lorenzo@hsr.it

In patients with type 1 diabetes, pancreatic β cells are destroyed by a selective autoimmune attack and their replacement with functional insulin-producing cells is the only possible cure for this disease. As of now, intrahepatic islet transplantation is the gold standard cell therapy for β cells replacement. The main limitations, however, remain the insufficient supply of human tissue and the need for lifelong immunosuppression therapy. Great effort is then invested in finding innovative sources of insulin-producing β cells. One old alternative with new recent perspectives is the use of non-human donor cells, in particular porcine β cells. Yet, large-scale production of human insulin-producing cells from stem cells is the most recent and promising alternative. Among the common unsolved problem, the lack of tissue engraftmet represents a relevant obstacle for a wide application of all these approaches in humans. We proposed, for the first time, the use of organ decellularization as a tool for the beta cell replacement strategy with the final goal to engineer a functional Vascularized Islet Organ (VIO). This approach demonstrated that the ex vivo combination of specific ECM native structure composition, geometry and the engraftment of the endocrine side of the pancreas in a dedicated vascular bed dramatically improved β cell survival, function and in vivo performance. This approach suggest a paradigm shift in the field: moving from an in vivo to an ex vivo engraftment strategy.

Keywords
beta cell; diabetes; engraftment

Abstract **#2252**

RECOMBINANT HUMAN BMP6 (RHBMP6) DELIVERED IN AUTOLOGOUS BLOOD COAGULUM IS A NOVEL THERAPY FOR BONE REGENERATION

Slobodan Vukicevic[P][1]

[1] *Laboratory for Mineralized Tissues, Center of Excellence for Regenerative Medicine, Center for Translational and Clinical Research, University of Zagreb School of Medicine*

Corresponding author's email: slobodan.vukicevic@mef.hr

We developed a novel autologous bone graft substitute (ABGS) implant composed of recombinant human BMP6 (rhBMP6) delivered in autologous blood coagulum (ABC). ABGS contains low doses of BMP6 as compared to BMP2 (InFuse®) and BMP7 (Ossigraft®) and is free of animal sourced collagen, not causing inflammation and immune responses. Safety and efficacy preclinical studies using rabbit ulna segmental defect model and posterolateral lumbar spine fusion models in rabbits and sheep have been conducted. ABGS was examined alone and in combination with allograft or synthetic ceramics as compression resistance matrices (CRM) in spine fusion models. ABGS induced a robust bone formation resulting in restoration of segmental defects and complete ectopic bone fusion with adjacent transverse processes at bilateral lumbar spine sites.

The safety and efficacy of ABGS was tested in Phase I/II randomized, double blind, placebo controlled trials in patients with a distal radius fracture (DRF) and high tibial osteotomy (HTO). ABGS was formulated to render a flexible and injectable implant. In the DRF study, three groups of patients were enrolled: standard of care, ABC alone and ABGS (ABC+rhBMP6). The HTO trial was conducted in 20 patients treated with 10 ml ABC containing placebo or 1 mg rhBMP6. Evaluation of ABGS with allograft is in progress following recruitment of 143 patients with degenerative disc disease to a randomized Phase II posterolateral lumbar interbody fusion (PLIF) study. Phase III trials will include patients with weight bearing long bone non-unions and transforaminal lumbar interbody fusion (TLIF) in patients with degenerative disc disease.

Abstract **#2253**

SCAFFOLDS FOR CARDIOVASCULAR TISSUE ENGINEERING VIA MELT ELECTROWRITING

Petra Mela[P][1]
[1] *Chair of Medical Materials and Implants, Department of Mechanical Engineering and Munich Institute of Biomedical Engineering, Technical University of Munich*

Corresponding author's email: petra.mela@tum.de

Introduction
Melt Electrowriting (MEW) is an advanced fiber forming additive manufacturing technology that has gained increasing attention for tissue engineering applications. Following a bioinspired approach for in situ cardiovascular tissue engineering, we developed fabrication strategies to obtain functional scaffolds with heterogenous architectures, controlled mechanical anisotropy and porosity.
Methods
A G-code generator was developed to output toolpath commands for the MEW machine based on user defined scaffold parameters. Different scaffold architectures were fabricated and investigated by scanning electron microscopy (SEM). Hybrid constructs could be obtained in combination with biologically favorable materials such as elastin and fibrin. Mechanical properties were determined by tensile testing, infiltration of human umbilical artery smooth muscle cells was verified after 1, 3, and 7 days of culture via SEM and (immuno)histology. Finally, tubular scaffolds were used for heart valves and were tested in a mock circulation system.
Results
MEW resulted in highly reproducible 3D scaffolds that closely matched the morphologies from the G-code generator. Complex heterogenous architectures were obtained, with fiber orientation and patterns defined by the user to tune the mechanical properties and anisotropy in a region-specific way. The scaffolds were fully infiltrated in vitro after 3 and 7 days. The functionality of heart valves based on the developed scaffolds was successfully verified according to the 5840 ISO requirements.
Conclusions
The versatile MEW platform enables the fabrication of highly tunable cardiovascular scaffolds with control on the architecture, heterogeneity, mechanical properties and porosity.

Abstract **#2254**

TARGETING SKELETAL CELL METABOLISM AS TISSUE ENGINEERING APPROACH

Geert Carmeliet[P][1]
[1] *KU Leuven*
Corresponding author's email: geert.carmeliet@kuleuven.be

Cell-based tissue engineering is a promising approach to treat large bone defects, but skeletal stem and progenitor cells are often lost during ex vivo expansion, resulting in reduced bone forming capacity. In addition, many cells do not survive after implantation because of insufficient supply of nutrients and oxygen, reducing thus the efficacy of this strategy. However, by targeting cell metabolism stem and progenitor properties can be preserved during ex vivo expansion and cells can be preconditioned to the nutrient-poor environment before implantation. Targeting cell metabolism during in vitro culture may thus improve the in vivo outcome of bone regeneration strategies.

Abstract **#2255**

MULTISCALE FRACTAL SHAPE CUES SUPPORT HIERARCHICAL ASSEMBLY AND MATURATION OF PODOCYTES VIA CURVATURE-INDUCED EXTRACELLULAR MATRIX PATTERNING

Anastasia Koroljₚ[1], Milica Radisic[1]
[1] *University of Toronto*
Corresponding author's email: anastasia.korolj@gmail.com

Nature efficiently self-organizes cells and tissues into complex fractal forms. Whether fractal patterning contributes functionally to maturation, and how cells sense and interpret such shape cues, is not well understood. Using kidney podocytes as a model system, bioinspired templating of glomerular histology was leveraged to design controlled fractal 2½ - D surfaces for cell culture. Microcurvature was associated with charge density gradients in space, found to direct extracellular matrix protein organization resulting in hierarchical assembly of cell structures and fractally-branching podocyte morphology in vitro, outlined with a novel fluorescent assaying technique. Shape simulation was uniquely associated with mature-like foot processes on an organized ECM. In applications of drug testing, coronavirus infection, and a cells-as-sensors approach to patient serum diagnostics, fractally stimulated cells were more responsive than flat cultures. Fractal frameworks may thus provide a functional role in podocyte maturation and could serve to advance other bioengineered systems.

Abstract **#2256**

UNRAVELLING METABOLIC DEPENDENCIES IN CANCER AND AUTOIMMUNITY

Celia Berkers[P][1,2]
[1] *Division Cell Biology, Metabolism & Cancer, Department Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University*
[2] *Biomolecular Mass Spectrometry and Proteomics group, Bijvoet Center for Biomolecular Research, Utrecht University*
Corresponding author's email: c.r.berkers@uu.nl

Dysregulation of metabolism contributes to many serious health problems, ranging from diabetes to cardiovascular disease and cancer. Changes in cellular metabolism are widely recognized as a hallmark of cancer cells and the rewiring of metabolism is considered essential for rapid tumour growth and proliferation. Because metabolic alterations aid in the cellular adaptation to pharmacologically changed environments, metabolic changes also play an important role in the development of drug resistance in cancer. In turn, cancer metabolism is intimately linked to immunity and immunometabolism. Upon activation, immune cells undergo a metabolic switch that is comparable to the metabolic rewiring observed in cancer cells. In the tumour microenvironment, immune cells compete with tumour cells for the same resources, further linker cancer and immuno-metabolism.
Interestingly, cells can become reliant on their metabolic reprogramming, a vulnerability that can be exploited for therapy. Research in the Berkers group aims to gain mechanistic insight in cancer and immuno-metabolism to ultimately identify novel metabolic targets for therapeutic intervention. In this talk, I will explain how we employ metabolomics and multi-omics strategies to study the role of metabolic pathways. I will showcase how metabolic inhibitors can be combined with chemotherapy to increase therapy responses. I will also highlight how omics methodologies can be used to shed light on T-cell metabolism.

Abstract **#2257**

IN TRANSITION TOWARDS SAFETY ASSESSMENT OF COMPOUNDS COMPLETELY BASED ON HUMAN DATA

Cyrille Krulp[1], Anne Kienhuis[2]
[1] *University of Applied Sciences Utrecht*
[2] *National Institute for Public Health and the Environment*
Corresponding author's email: cyrille.krul@hu.nl

Safety and efficacy testing of compounds is traditionally performed in laboratory animals, with limitations regarding translatability to humans. For the development of better translational methods we need advanced in vitro models that closely mimic the human situation. Such (organ-specific) models based on human cells are preferred over animals and immortalized cell lines. Recent scientific developments in tissue engineering, microfluids and material science have led to the development of promising advanced in vitro models, such as organ-on-a-chip and organoids. An advantage of using these type of models is that they provide more mechanistic information on chemical-induced adverse effects on biological pathways. For applications in toxicology, especially in the regulatory context and the acceptance and use by industry, performance and acceptance criteria need to be defined. These criteria include requirements with respect to the validity (relevance, robustness, reproducibility, reliability), transparency and accessibility. Furthermore, the context-of-use of advanced in vitro models need to be defined for complex technology such as organ-on-a-chip. 'As simple as possible and as complex as necessary', seems to be a good starting point. Recently we started with the development of a 'Virtual Human Platform': a new approach integrating existing human data, data obtained from in vitro models, and exposure assessment with AI/machine learning. We aim to design a new framework for safety assessment of chemicals. This should be a combined effort of all stakeholders, researchers, industry, regulators, NGO's and patients to address the emerging challenge to accelerate the transition towards animal-free safety assessment completely based on human data.

Keywords
transition; safety assessment ; virtual human

References
The Virtual Human Platform for safety assessment (VHP4Safety) NWA project 1292.19.272, funded by NWO-ORC

Abstract **#2258**

DE HUMANI CORPORIS FABRICA: ORGANOID-BASED MODELING FOR PERSONALIZED DISEASE TRAJECTORIES AND INTERCEPTIVE MEDICINE

Giuseppe Testa[P][1]
[1] *Fondazione Human Technopole*
Corresponding author's email: giuseppe.testa@ieo.it

Session keynote invitee, not applicable

Abstract **#2259**

THE FABRIC OF LIFE: HOW FIBRE SYSTEMS WITH UNIQUE PROPERTIES DETERMINE TISSUE MECHANICS AND FUNCTION

Rudolf E. Leube[P][1]
[1] *RWTH Aachen University*
Corresponding author's email: rleube@ukaachen.de

The most abundant polypeptides produced by the organism form elongated polymer chains, which assemble into complex fibre systems. Their diversity and plasticity support all of life's functions and are essential for tissue morphogenesis and maintenance. An important aspect is the physical connectivity between the different intra- and extracellular fibre systems defining tissue mechanics.
We study the cytoplasmic keratin intermediate filament-based epithelial cytoskeleton as a paradigm to examine these relationships. The more than 50 keratins form highly heterogeneous filaments with an epithelial cell type- and context-dependent composition. The compositionally unique filaments assemble into different types of cytoplasmic networks, which reflect and determine the cross talk between epithelial differentiation and function. The keratin cytoskeleton is linked to the nuclear cytoskeleton and to the extracellular matrix through different types of protein assemblies. Together, these fibre systems co-ordinate cell and tissue behaviour controlling homeostasis at different length and time scales.

Abstract **#2260**

BIOMEDICAL APPLICATIONS OF MAGNETIC NANOPARTICLES: REMOTE CONTROL OF CELLS

Jon Dobson[P][1]
[1] *University of Florida*
Corresponding author's email: jdobson@ufl.edu

The use of magnetic micro and nanoparticles for biomedical applications was first proposed in the 1920s as a way to measure the rheological properties of the cytoplasm. Since that time, particle synthesis techniques and functionality have advanced significantly. Magnetic micro and nanoparticles are now used in a variety of biomedical applications. More recently, magnetic micro and nanoparticles have been used to investigate and manipulate cellular processes both in vitro and in vivo.

This talk will focus on our work developing Magnetically Activated Receptor Signaling (MARS) – a magnetic nanoparticle–based technique for activating cell surface receptors and controlling the activity of biomolecules such as growth factors. The basic principles involve surface functionalization of magnetic nanoparticles (MNPs) with molecules targeting specific cell surface receptors. We have investigated antibodies targeting specific ion channels (e.g. TREK1), surface receptors (e.g. PDGFRα & β) and peptides (e.g. RGD) targeting integrins. The particles bind to the receptors and, upon the application of a highgradient external magnetic field, energy is transferred to the particles. The energy delivery induces a conformation change in the receptor, activating the specific biochemical signaling pathway associated with that receptor. By targeting specific receptors, we have been able to control ion channel activity, activate bone and cartilage matrix formation, and control the differentiation of human mesenchymal stem cells both in vitro and in vivo. We have developed this technology for applications in cell engineering/regenerative medicine and stem cell therapy. The MARS technology has applications in regenerative medicine, drug screening and cell engineering.

Keywords
magnetic; nanoparticles; regenerative medicine

Abstract **#2261**

STUDYING CARDIAC SIDE EFFECTS OF SARS-COV-2 INFECTION AND SCREENING EXTRACELLULAR VESICLE THERAPEUTICS IN VITRO

Karl Wagner[P][1,2], Milica Radisic[1,2,3]
[1] *Department of Chemical Engineering and Applied Chemistry, University of Toronto*
[2] *Institute of Biomedical Engineering, University of Toronto*
[3] *Toronto General Research Institute, University Health Network*
Corresponding author's email: karl.wagner@mail.utoronto.ca

Unlike seasonal coronaviruses, SARS-CoV-2 has a profound tropism for the heart. Estimates indicate 78% of individuals infected with SARS-CoV-2 experience cardiac side effects, with asymptomatic individuals still at risk of viral-induced heart failure, yet the mechanisms and consequences of such effects remain unclear. [1] Thus, therapeutics targeting SARS-CoV-2-induced heart failure remain elusive. The organ-on-a-chip industry has emerged at the intersection of microfluidics and tissue engineering, combining cells and biomaterials in arrangements that mimic organ processes, facilitating investigation of human physiology in a controlled and accessible environment. [2],[3] Recent studies indicate that cell signalling in the heart plays an integral role in tissue physiology and phenotype. [4],[5] For instance, it has been suggested that extracellular vesicles (EVs) released and taken up by cells in the heart are critical to regulating cardiac function and cellular responses to stress, disease, and injury. [6],[7] The “Biowire” model of cardiac tissue-on-a-chip was used to study the cardiac side effects of coronavirus infection in the heart and to screen EV therapeutics for mitigating such effects. EVs sourced from induced pluripotent stem cells (iPSCs) facilitated the recovery of infected cardiac tissue function to baseline levels. miRNA sequencing and gene ontology analyses suggested several stress responsive pathways are targeted by iPSC-EV miRNA that may alleviate some detrimental effects of coronavirus infection. Limited knowledge regarding SARS-CoV-2 side effects in the heart make tissue-on-a-chip models a novel tool to better understand the mechanisms of viral-induced heart failure and to study the potential for cell signalling-based therapeutics to improve patient outcomes.

Keywords
extracellular vesicles; SARS-CoV-2; cardiac tissue-on-a-chip

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Abstract **#2262**

MUSCLE AND FAT CELL MATURATION ON EDIBLE 3D-PRINTABLE SCAFFOLDS FOR CULTURED MEAT DEVELOPMENT

Shulamit Levenberg[P][1]
[1] *Technion-Israel Institute of Technology*
Corresponding author's email: Shulamit@bm.technion.ac.il

Cultivated meat is a promising technology that could generate meat without the need for animal agriculture. Generating a tissue requires a 3D scaffold to provide support to the cells and mimic the extra cellular matrix. The scaffolding materials must meet key requirements to enable cell growth and maturation, and should preferably have nutritional values. In our research we developed nutritious scaffolding compositions which enable flexible fabrication processes of edible bovine muscle and fat tissue. The biomaterials were assessed both for 3D-printing capabilities and for their support of growth and maturation of muscle-fibers and fat cells. The suggested 3D-printable compositions enable a cell-friendly fabrication of nutritious scaffolds in well defined geometries, capable of supporting cell growth and maturation, as a step towards an edible engineered steak.

Abstract **#2263**

CULTURED MEAT: HYPE AND REALITY

Gabor Forgacs$_P$[1,2]
[1] *University of Missouri-Columbia*
[2] *Fork & Goode, Inc.*
Corresponding author's email: gabor@forkngoode.com

Any attempt of making cultured meat needs to deal with three fundamental issues: choice of cells, nutrient medium needed to grow the cells and the bioprocess, the particular way to grow cells. The good news is that the scientific and technical methods to tackle these issues are known; no new rocket science is needed. The sobering news is that none of these methods have been tested at the scale where the cultured meat industry needs to operate. To put things in perspective: 1 kg biomass (~10 hamburgers) contains about quarter trillion cells and requires liters of medium to culture, in massive bioreactors. Furthermore, estimating the cost associated with the running of such operation and comparing it with the Walmart price of about $6/kg of ground meat evidence that significant practical challenges exist for the marketing of cultured meat. Academics never deal with scale issues, whereas this nascent industry is all about scale. Commercial cultured meat activity started in 2011 and by now there are more than 100 companies in the space. Investment is growing at an impressive pace. When early progress is so spectacular, it is tempting to make statements that unavoidably fuel hype. The aim of this talk is to draw attention to the existing challenges, emphasize that they are solvable and call all the interested parties to participate in solving the challenges. This might be particularly appropriate at this forum, with many tissue engineers who are dealing with many of the same challenges as the cultured meat community.

Keywords
cultured meat; cell culture; bioreactor

Abstract **#2264**

IMMUNOBIOLOGY AS A DETERMINANT OF SUCCESSFUL REGENERATIVE MEDICINE

Stephen Badylak[P][1,2]
[1] *University of Pittsburgh*
[2] *McGowan Institute for Regenerative Medicine*
Corresponding author's email: badylaks@upmc.edu

Whereas the primary function of the immune system was long considered to be defense against potential pathogens, discoveries during the past twenty years have established the critical role that the immune system plays in fetal development, homeostasis in healthy tissues, the host response to tissue injury, and the continuous crosstalk that occurs between the immune system and the stem/progenitor cells. These findings have fundamentally changed tissue engineering/regenerative medicine (TE/RM) strategies to include immunomodulatory biomaterials, nanovesicles as theranostic tools, and the delivery of specific signaling molecules that target regulatory and effector cells to promote functional tissue repair and reconstruction. Cross disciplinary approaches between the TE/RM and immunology fields have never had greater potential to change the practice of medicine.

Keywords
immunobiology

Abstract **#2265**

RETROSPECTIVE ANALYSIS OF ENHANCER ACTIVITY AND TRANSCRIPTOME HISTORY; DEFINING CELL SIGNALING PATHWAYS DIRECTING DEVELOPMENT AND CELL DIFFERENTIATION.

Joost Gribnau[P][1,2]
[1] *Erasmus MC*
[2] *Oncode Institute*
Corresponding author's email: j.gribnau@erasmusmc.nl

The development of more than 300 cell types present in our body requires the action of complex molecular mechanisms. These involve activation of signal transduction pathways that instruct transcription factor networks and the epigenetic landscape to generate the gene expression programs that dictate the cell state. The development of the iPS technology provides a unique opportunity to drive differentiated cell types back in development and generate embryonic stem cells of virtually every patient, to study developmental control, homeostasis, and model disease. Unfortunately, robust and efficient protocols to differentiate these iPSCs to a specific cell type are lacking, and therefore increased knowledge of the mechanisms directing embryonic development is key to be able to establish proper in vitro differentiation models systems. In my lecture I will describe the DCM-time-machine (DCM-TM) technology we developed to establish gene and enhancer activity maps of the past and predict cell signaling pathways that drive differentiation of a stem cell to a differentiated cell type. DCM-TM utilizes an inducible DCM-RNA-polymerase-subunit-b fusion protein, to label active genes and enhancers with a bacterial methylation mark that does not affect gene transcription and is propagated in S-phase. We applied DCM-TM to study intestinal homeostasis providing new insights in cell signaling, and chromatin and transcription factor dynamics in lineage fate decisions. DCM-TM can be applied to follow cell differentiation, embryonic development and tissue regeneration, identifying temporal maps of transcription factor networks and signal transduction pathways that can be used to improve stem cell expansion and cell differentiation models.

Keywords
Stem cell; Lineage tracing; Differentiation

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Abstract **#2266**

SMALL TRICKS TO MEND A BROKEN HEART

Leon de Windt[P][1]
[1] *Maastricht University*
Corresponding author's email: l.dewindt@maastrichtuniversity.nl

Myocardial regeneration is restricted to early postnatal life, when mammalian cardiomyocytes still retain the ability to proliferate. The molecular cues that induce cell cycle arrest of neonatal cardiomyocytes towards terminally differentiated adult heart muscle cells remain obscure. Here we report that a microRNA cluster is higher expressed in the heart of newborns and decreases in expression towards adulthood, especially under conditions of overload, and orchestrates the transition of cardiomyocyte hyperplasia towards cell cycle arrest and hypertrophy by virtue of its targetome. Taking advantage of the regulatory function of this microRNA cluster on cardiomyocyte hyperplasia and hypertrophy, viral gene delivery provoked near complete regeneration of the adult myocardium after ischemic injury. Our data demonstrate that exploitation of conserved molecular programs can enhance the regenerative capacity of the injured heart.

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Abstract **#2267**

PLURIPOTENT STEM CELL CULTURE, DIFFERENTIATION AND SORTING ON THERMORESPONSIVE SURFACE

Akon Higuchi[P][1]
[1] *National Central University*
Corresponding author's email: higuchi@cc.ncu.edu.tw

Human induced pluripotent stem cells (hiPSCs) and embryonic stem cells (hESCs), which are described as human pluripotent stem cells (hPSCs), are attractive for stem cell therapy [1] and are used for cardiac studies of (a) cardiac disease modeling, (b) cardiotoxicity screening, (c) drug discoveries, and (d) human cardiac development [2]. The development of efficient high production protocols with high purity for hPSC induction into cardiomyocytes is demanded in recent research. The current differentiation process of hPSCs into cardiomyocytes to enhance the purity of hPSC-derived cardiomyocytes requires some purification processes, which are laborious processes. We developed cell sorting plates, which are prepared from coating thermoresponsive poly(N-isopropylacrylamide) and extracellular matrix proteins [3]. After hPSCs were cultured and induced into cardiomyocytes on the thermoresponsive surface coated with laminin-521 for 15 days, the temperature of the cell culture plates was decreased to 8-9 ℃ to detach the cells partially from the thermoresponsive surface. The detached cells exhibited a higher cardiomyocyte marker of cTnT than the remaining cells on the thermoresponsive surface as well as the cardiomyocytes after purification using conventional cell selection. The detached cells expressed several cardiomyocyte markers, such as α-actinin, MLC2a and NKX2.5. This study suggested that the purification of hPSC-derived cardiomyocytes using cell sorting plates with the thermoresponsive surface is a promising method for the purification of hPSC-derived cardiomyocytes without conventional laborious processes.

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Abstract **#2268**

IN-VIVO-INSPIRED ENVIRONMENTS TO UNDERSTAND CANCER PLASTICITY

Peter Friedl[P][1]
[1] *Radboud University Medical Centre*
Corresponding author's email: peter.friedl@raboudumc.nl

Analysis of the metastatic cascade in defined environments requires to account for multi-parametric mechanical and chemical determinants of the tumor and stromal cells as well as cell deformability and adhesion regulation relative to the 3D tissue topologies. When monitored in vivo, using intravital multiphoton second and third harmonic generation and fluorescence microscopy, tissue microniches provide invasion-promoting tracks that enable collective migration along tracks of least resistance. As main routes, non-destructive contact-guidance is mediated by preformed multi-interface perimuscular, vascular and –neural tracks of 1D, 2D and 3D topography. 3D ultrastructural analysis reveals predefined tissue conduits ("highways") of defined geometry, nanotropography and molecular composition as predominant routes of invasion by contact guidance combined with a cell "jamming" mechanism (1). Using this in vivo data, we have developed 3D collagen-based interface models which reliably predict the basic programs of cancer cell invasion as well as their adaptive plasticity response after molecular intervention (2). The 3D in-vivo-inspired environment reliably predicted tumor plasticity and escape after therapeutic targeting of beta1/beta3 integrins as well as in response to hypoxia, including collective and amoeboid single-cell dissemination, which was followed by enhanced metastasis. In conclusion, interstitial tissue models for modeling the metastatic cascade need to account for the physicochemical programs that balance cell-intrinsic adhesion and mechanocoupling with encountered physical space and molecular cues. The combination of different imaging techniques is critical to dissect cancer tissue dynamics, as well as derive guidance and jamming/unjamming principles of morphogenesis and tissue regeneration.

Keywords
Invited by Lorenzo Morini for the Frontiers Symposium

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Abstract **#2269**

BIOPRINTED AUTOLOGOUS SKIN SUBSTITUTE: PRECLINICAL STUDIES AND DEVELOPMENT OF A GMP-COMPLIANT BIOPRINTER

Fabien Guillemot[P][1], Laurence Hutter[1], Jérémy Magalon[2], Julie Véran[2], Florence Sabatier[2], Maxime Abellan Lopez[2]
[1] *Poietis*
[2] *Marseille Hospital (AP-HM)*
Corresponding author's email: contact@poietis.com

Main challenges for the manufacturing of tissue engineered advanced therapy medicinal products (ATMPs) relate to the standardisation of manufacturing processes and the improvement of tissue functionality, and cost-effectiveness and profitability of related treatments. To tackle these challenges, Poietis has been developing the Next-Generation Bioprinting (NGB) platform. NGB-C bioprinter has been designed to be GMP-compliant and integrates automation and robotics technologies, as well as the main bioprinting techniques (laser-assisted bioprinting and bioextrusion). Using NGB-C, an autologous dermo-epidermis skin substitute, Poieskin, is developed as the first therapeutic bioprinted tissues. In this presententation, we will introduuce results obtained during preclinical studies.

Abstract **#2270**

GENE THERAPY APPROACHES IN THE AUSTRIAN CLUSTER FOR TISSUE REGENERATION

Heinz Redlp[1,2,3], Amelie Frischer[1,3], Simon Sperger[1,3], David Hercher[1,3], Johannes Grillari[1,3]
[1] *Ludwig Boltzmann Institute for Traumatology in the AUVA Research Center, Donaueschingenstrasse 13, 1200 Vienna*
[2] *Ludwig Boltzmann Research Group SHoW – Senescence and Healing of Wounds, Donaueschingenstrasse 13, A-1200 Vienna, Austria*
[3] *Austrian Cluster for Tissue Regeneration*
Corresponding author's email: heinz.redl@trauma.lbg.ac.at

One option to improve tissue regeneration is the use of growth factors. As a promising alternative to recombinant protein therapy, gene therapy allows local and sustained release of growth factors. This moderate and constant release is more suitable for regenerative processes compared to administration of high protein doses. Exogenous growth factors are produced by cells in situ; such highly bioactive amounts of growth factors are produced directly at the defect site, thereby highly limiting adverse off-target effects.
Targeted research areas include (combinatorial) gene therapy approaches for bone/musculoskeletal tissue regeneration in vivo and ex vivo. Amongst them, new techniques such as viral and non-viral gene delivery systems, next-generation therapeutical DNA vectors for example with decreased immunogenicity, enhanced bioactivity of growth factor and enhanced gene expression. Beside DNA based gene therapy also mRNA based technologies are increasingly used and becoming popular by Covid vaccination successes.
Increasing records of clinical success in the last years have constantly improved awareness of gene therapy, strengthening the enthusiasm of the community for novel and effective treatment methods providing the needed momentum for further developments.

Abstract **#2271**

TRANSLATIONAL APPROACHES IN THE AUSTRIAN CLUSTER FOR TISSUE REGENERATION

Heinz Redl[1,2,3]
[1] *Ludwig Boltzmann Institute for Traumatology in the AUVA Research Center, Donaueschingenstrasse 13, 1200 Vienna*
[2] *Ludwig Boltzmann Research Group SHoW – Senescence and Healing of Wounds, Donaueschingenstrasse 13, 1200 Vienna, Austria*
[3] *Austrian Cluster for Tissue Regeneration*
Corresponding author's email: heinz.redl@trauma.lbg.ac.at

Bringing translational research to TRL 5-7 requires a lot of resources including regulatory input with International Organization for Standardization (ISO) studies under good laboratory practice (GLP) and good manufacturing process (GMP) conditions and cannot be completed through academic institutes alone. It is usually difficult to achieve this through grant funding only, therefore the formation of Start-up companies or cooperation with established companies is necessary. While the initial phase of initiating a spin-off is quite well supported in many countries, raising money thereafter can be difficult and some of the SMEs do not survive for long. In the Austrian Cluster for Tissue Regeneration we have 14 Spin-off companies both on the material side (Lithoz, UpNano, CubiCure, MorphoMed, THT-Biomaterials) and human cells/products (Evercyte, Liporegena, Phoenestra) as well as a in vitro and in vivo preclinical models (Trauma Care Consult, Pregenerate, Saico Biosystems). We will give examples how different companies evolved in the field and highlight the challenges faced when translating research to the clinical and further on to the commercial field.

Keywords
Session: Scaling up and maturation in tissue engineering

Abstract **#2272**

DISSECTING ENDOTHELIAL-STROMAL INTERACTIONS IN A 3D TUMOUR MICROENVIRONMENT MODEL

Laura Bray[P][1]
[1] *Queensland University of Technology*
Corresponding author's email: laura.bray@qut.edu.au

The tumour microenvironment is a key contributor to cancer development and progression. Reciprocal interactions between the epithelium and the adjacent stroma are essential for malignant transformation. Cancer-associated fibroblasts (CAFs) are a fundamental component of the tumour stroma. CAFs contribute to cancer progression through altered paracrine signalling as well as by remodelling of the ECM. The arising aberrant biochemical, biomechanical, and topographical cues promote invasiveness and migration of tumour cells. It is crucial to consider stromal and endothelial features in in vitro cancer models to understand the role of the tumour microenvironment in cancer progression. In this research, bioengineered experimental models provided a more physiologically relevant platform for tumour angiogenesis research in vitro. Primary tissue-specific endothelial and fibroblast cells (non-malignant fibroblasts (NFs) and CAFs) were used to study differential influences of these cell types on tumour angiogenesis and epithelial plasticity within a tissue-specific context. Distinct biomechanical and cytoskeletal characteristics of patient-matched NFs and CAFs were elucidated. Utilising a co-culture model, a decreased cellular stiffness was found in benign epithelial cells grown in the presence of CAFs, which coincided with a more invasive and proliferative phenotype. To mimic early cancer angiogenesis in vitro, tissue-specific microvascular cells were cultured in the presence of NFs or CAFs within 3D semi-synthetic hydrogels. Cell heterogeneity within the tissue-specific tumour angiogenesis model was assessed using high-throughput techniques. Overall, this works contributes to the understanding of the tumour microenvironment by providing novel insights into the differential characteristics between the healthy and the malignant tumour microenvironment.

Abstract **#2273**

CURRENT PERSPECTIVES, CHALLENGES AND ADVANCES IN REGENERATIVE MEDICINE THERAPIES

Julie Allickson[P][1]
[1] *Mayo Clinic Center for Regenerative Medicine,*
Corresponding author's email: allickson.julie@mayo.edu

Regenerative Medicine is an extremely broad division of medicine that can repair, replace, or regrow cells, tissues, or organs. The technology can include cells, genes, biomaterials, or scaffolds. Despite the encouragement of preclinical studies, small early-phase clinical trials and record-breaking financial investment in the field, we are not observing many approved regulated therapies. The first six months of 2021 demonstrated the greatest investment in the field, raising more than $14 billion reaching greater than 70% of all of 2021's $19.9 billion dollar investment (1). FDA and other regulatory agencies have worked hard to be transparent on regulatory requirements with heightened education (2). The field has increased educational opportunities in Regenerative Sciences including a PhD program at Mayo Clinic. 2021 was the first-time gene-editing technology was approved for clinical trials using CRISPER to treat amyloidosis (3). Although exciting technology advancements move to clinic, the field is plagued with challenges that do not allow regenerative medicine to be standard of care. Manufacturing is and has been a roadblock including scaling the therapy, cost of therapies, well planned and executed clinical trials including objective endpoints, biological variability, and product reimbursement. There is a need for translational guidance, multi-disciplinary teams, and increased understanding of product functionality.

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Abstract **#2274**

INDUSTRIALIZATION OF REGENERATIVE MEDICINE

Laura Niklason[P][1]
[1] *Humacyte, Inc.*
Corresponding author's email: Niklason@humacyte.com

Scientific and medical understanding are essential for bringing regenerative medicine products to the clinic. Furthermore, translation of regenerative medicine products requires cutting-edge technology, scaled manufacturing, and clinical trial and regulatory expertise. Since its founding in 2005, Humacyte has worked to sequentially solve the translational hurdles related to the production of Human Acellular Vessels, or HAVs. Humacyte's platform involves the use of differentiated human vascular cells that are cryopreserved and are leveraged to generate engineered vascular tissues of a shape and size dictated by the bioreactor and polymer scaffolding of our manufacturing process. To date, HAVs have been implanted into more than 450 patients having diseases ranging from kidney failure, to peripheral arterial disease, to vascular trauma. Recent progress in commercial-scale manufacturing has produced a system that can produce HAVs in batches of 200, in a highly automated and controlled fashion. Recent clinical data has shown that these HAVs produced in the commercial-scale manufacturing system function as expected when used as dialysis access grafts. In addition, smaller caliber HAVs that are produced in versions of the commercial system are being studied in pre-clinical models of cardiac surgery, showing the broad applicability of the HAV platform. Progress in clinical evaluations, combined with implementation of commercial-scale manufacturing, both contributed to Humacyte's ability to transition to the public markets in August of 2021.

Keywords
Bioengineering; Biotechnology; commercial-scale manufacturing

Abstract **#2275**

CRACKING OPEN THE BIOFABRICATION WINDOW

Marcy Zenobi-Wong$_P$[1]
[1] *ETH Zürich*
Corresponding author's email: marcy.zenobi@hest.ethz.ch

Bioprinting has been proposed as a disruptive technology to revolutionize the tissue engineering field by enabling the production of more accurate, more complex and more functional tissues. The field has made rapid advances in both the development of bioprinting materials as well as the introduction of new bioprinting technologies. However, the challenge of printing of large-scale tissues which still embody key nano- and micro-scale features might be only possible through the convergence of individual printing technologies and their respective materials. In recent years, there has been much work to identify 'sweet spots' in which both good printing resolution and biocompatibility/bioactivity are possible. For certain techniques, such as extrusion bioprinting, the use of a finer nozzle to improve resolution is inherently in contradiction with improved viability due to the increase shear stress. Here new non-contact or nozzle-free methods could play an important role. Of these, printing methods which address all points in the model simultaneously may enable a larger 'sweet space' in which speed, resolution and excellent cell viability are possible.

Abstract **#2276**

BIOENGINEERING 3D TUMOUROIDS TO INTERROGATE THE TUMOUR-STROMA BOUNDARY

Umber Cheema[P][1]
[1] *UCL*
Corresponding author's email: u.cheema@ucl.ac.uk

In vitro models of solid tumours are being developed to further elucidate mechanisms of tumour growth, invasion and metastasis. These models are useful tools to measure the efficacy of specific therapeutic interventions and can be used as platforms to test novel drugs. Current model systems used include spheroid culture, matri-gel cultures, Organ on a chip and organoids. We have developed 3D tumouroids as a biomimetic model with the specific aim to bioengineer the tumour and stroma component with a distinct boundary to allow for precise quantification of cancer invasion. Dependent upon the tissue of interest, we have bioengineered connective tissue stroma and bone stroma, along with engineered primitive vascular networks to enhance tissue biomimicry. As tumouroid models are further developed to add tissue complexity, and thus become more biomimetic, they will eventually replace early testing within in vivo models.

Abstract **#2277**

MULTI-LAYERED POLYMER SHEETS: A TOOL TO TACKLE CORNEAL DISEASES

Jasper Delaey$_P$[1], Jasper Van Hoorick[1], Bert Van Den Bogerd[2], Carina Koppen[2], Peter Dubruel[1], Sandra Van Vlierberghe[1]

[1] Polymer Chemistry & Biomaterials group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent (Belgium)

[2] Department of Ophthalmology, Visual Optics and Visual Rehabilitation, Faculty of Medicine and Health Sciences, University of Antwerp, Wilrijk (Belgium)

Corresponding author's email: jasper.delaey@ugent.be

Corneal endothelial damage and diseases are two of the major contributors to blindness or severe visual impairment worldwide (1). The corneal endothelium is the innermost cell layer of the cornea and consists of a cellular monolayer that maintains the stroma in a dehydrated state through a “pump-and-leak” mechanism. A critical loss of cells due to damage, disease or aging leads to corneal edema which in turn results in opacification of the cornea (2). Currently, the only treatment consists of a corneal transplantation from healthy cadaveric donor tissue. Unfortunately, only 1 donor is available for every 70 cases (3). To tackle this shortage, the present work focusses on the development of transparent (>90%, 380-700nm), ultra-thin (≤5µm), multilayered sheets that are permeable towards glucose (Papp >2.36*10-3cm/s). These sheets constitute a poly(D,L-lactide) (PDLLA) layer to provide structural rigidity (E=4.95 ± 0.81MPa) and a crosslinkable gelatin-based hydrogel (Gel-MA-AEMA (4)) as an extracellular matrix (ECM) mimic. These sheets provide a substrate for corneal endothelial cells (CECs) (primary human CECs and B4G12 immortalized CECs) to enable subsequent ocular implantation with the aim to restore the damaged endothelium and the patient’s vision (5). Successful membrane production and coating were verified using X-ray photoelectron spectroscopy (XPS) and static contact angle measurements. Cells seeded on the membranes showed their characteristic hexagonal morphology, the presence of Na+/K+ ATPase pumps and tight junctions (ZO-1) as evidenced using immunocytochemical staining. Additionally, research has been devoted towards upscaling of the membranes while ensuring straightforward tunability of the thickness, through doctor blading.

Keywords

Gelatin; Corneal endothelium

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Abstract **#2278**

IMPROVED OSTEOINDUCTION BY NON-VIRAL GENE THERAPY USING COMBINATIONS OF BMPS

Amelie Frischer[P][1,2], David Hercher[1,2], Darja Marolt[1,2], Heinz Redl[1,2], Johannes Grillari[1,2], Ara Hacobian[1,2]
[1] *Ludwig Boltzmann Institute for Traumatology, Vienna, Austria*
[2] *Austrian Cluster for Tissue Regeneration, Vienna, Austria*
Corresponding author's email: amelie.frischer@trauma.lbg.ac.at

In the field of bone regeneration, the TGFβ-superfamily of growth factors contains candidate proteins offering the possibility to improve the healing process. Belonging to this family of proteins, bone morphogenetic proteins (BMPs) have been studied intensively in respect to their involvement in bone development.
Several BMPs are known for their osteoinductive activity - BMP-2, -4, -6, -7, and -9 constituting the most promising candidates. In clinics, BMPs are applied as recombinant proteins. A major bottleneck is the need for relatively high doses and repeated administration. Gene therapy constitutes an attractive alternative, not only because of lower production costs when compared to recombinant proteins, but also in terms of efficiency by enabling locally restricted and prolonged production of the respective proteins.

In their active form, BMPs can act as either homodimers or heterodimers. It has been reported that heterodimers exhibit a higher potential to induce osteogenic differentiation.
In this study, sequence optimized versions of BMP-2, -4, -6, -7 and -9 were cloned into gene expression vectors under the control of a constitutive EF1α-promoter. Subsequently, different BMP combinations were analysed regarding their capability to induce osteogenesis in transfected mouse myoblast C2C12 via alkaline phosphatase activity assay (ALP) and realtime-PCR.
Results showed significantly improved osteoinduction compared to transfection with single BMPs. In addition to the already known strong osteoinductive capacity of BMP-2 and -7 heterodimers we identified several equally potent combinations of BMPs. Interestingly, single transfection with BMP-9 showed similar osteoinduction.

Keywords
Gene Therapy; Bone Morphogenetic Proteins; Osteogenic Induction

Abstract **#2279**

A RETINOIC ACID ANALOG AND CORRECT TIMING OF BMP2 IMPROVES THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO ARTICULAR CARTILAGE

Fabrizio E Mancini[P][1], Marco Domingos[2], Leela Biant[3], Susan Kimber[1]

[1] *Division of Cell Matrix Biology and Regenerative Medicine University of Manchester, Manchester, UK.*

[2] *Department of mechanical, Aerospace and Civil Engineering, University of Manchester, Manchester, UK.*

[3] *Deparment of Trauma & Orthopaedic Surgery, Manchester University Hospitals Foundation Trust, Manchester, UK*

Corresponding author's email: fabrizio.mancini@postgrad.manchester.ac.uk

Osteoarthritis is the most widespread degenerative condition of joint cartilage leading to chronic pain and immobility. The lack of drug therapies providing tissue restoration and the relatively short lifespan of joint-replacement implants, indicates the need for an articular cartilage regeneration strategy. Articular cartilage is derived from lateral plate mesoderm and limb bud during development. Our group established protocols for the differentiation of pluripotent human embryonic stem cells (hESCs) to osteochondral progenitors [1-2]. TTNPB (4-[(E)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid), a retinoic acid receptor agonist, has been reported to induce chondrogenesis in stem cells [3]. Analysis by qRT-PCR showed that addition of TTNPB to our previous protocol significantly improved the expression of SOX9 and COL2A1 while reducing expression of hypertrophic markers such as COL1A1 and RUNX2. Although the reported protocol could provide a promising and robust cell source for better understanding the mechanism of articular joint development in humans, off-target subpopulations may form during the process of differentiation, hindering further application of this protocol. In this regard, we developed a CRISPR-Cas9-edited hESC line with a COL2A1-SOX9 response element reporter to select cells in combination with reported chondrogenic cell surface markers such as BMPR1b, CD146 and CD166. We are now evaluating their differentiation potential in comparison to non-selected cells. The implementation of a cell sorting strategy based on markers for the discrimination of chondro- and osteo-progenitors would improve the potential and safety of our protocol. When fully characterised by RNA-seq, this protocol could lay the foundations for possible cell therapy strategies for osteoarthritis.

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Abstract **#2280**

ENGINEERING THE BIOMATERIAL INTERFACE FOR TRANSLATIONAL REGENERATIVE MEDICINE

Molly M Stevens[P][1]
[1] *Imperial College London*
Corresponding author's email: m.stevens@imperial.ac.uk

This talk will provide an overview of our recent developments in bio-instructive, self-assembling and gradient materials for applications in regenerative medicine with focus on establishing translational pipelines to bring our innovations to the clinic [1]. I will present how we are using remote fields to engineer complex 3D architectures that mimic anisotropic and multiscale tissue structures and generate spatially arranged bioinstructive biochemical cues, such as acoustic stimulation to produce engineered muscle with bundles of aligned fibres [2]. I will discuss recent developments in our tunable nanoneedle arrays for multiplexed intracellular biosensing at sub-cellular resolution and modulation of biological processes [3], and will talk about our portfolio of nanoparticle-based sensing probes for disease monitoring in vivo such as renal clearable gold nanoclusters for cancer detection [4]. Finally, I will present our advances in Raman spectroscopy characterisation techniques for high-throughput label-free characterization of single nanoparticles (SPARTA) which is becoming an integral tool for the design of advanced nanotherapeutics [5]. I will explore how these versatile technologies can be applied to transformative regenerative medicine innovations.

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Abstract **#2281**

A 3D BIOPRINTED MODEL OF THE HUMAN INTERVERTEBRAL DISC

Sam R Moxon[P][1], Marco Domingos[1], Julie Gough[1], Stephen M Richardson[1]
[1] *University of Manchester*
Corresponding author's email: s.richardson@manchester.ac.uk

The intervertebral disc (IVD) aids the flexible movement of the spinal column by providing vertebra with support against mechanical loads. At its core, the IVD contains a soft and gelatinous nucleus pulposus (NP) populated by spherical NP cells embedded in a polysaccharide-rich matrix. Conversely, the surrounding annulus fibrosus (AF) is much stiffer and comprised of elongated AF cells in an aligned, collagenous matrix. Age-related IVD degeneration leads to chronic pain and can result in permanent disability. IVD repair is impaired by an inability to regenerate the two distinct regions of the disc simultaneously. In this study a newly developed suspended hydrogel (SLAM) bioprinting system was employed to fabricate IVD analogues for tissue regeneration and modelling of developmental and degenerative processes. Constructs were fabricated with distinct regions of gellan gum polysaccharide and type I collagen in order to mimic the structural and mechanical gradients within IVD tissue microenvironments. Moreover, cell-laden IVD constructs were bioprinted without compromising cell viability. Human NP cells embedded within the central “NP-like” gellan gum region of the construct retained a spherical morphology resembling what is observed in vivo. Within the outer collagenous “AF-like” region, human AF cells exhibited an elongated morphology and showed evidence of regions of cell alignment. Further inspection of these regions with transmission electron microscopy revealed the presence of aligned collagen fibres. These data highlight the potential to utilise SLAM for the generation of constructs that can reflect the heterogeneous nature of the IVD for disease modelling and regenerative medicine applications.

Keywords
Intervertebral disc; Bioprinting

Abstract **#2282**

PRODUCTION OF UROCORTIN-1 LOADED NANOPARTICLES FOR CHONDROPROTECTION APPLICATIONS

Zara Smith[P][1], Annalisa Tirella[1], Paul Townsend[1,2], Stephen M Richardson[1]
[1] *University of Manchester*
[2] *University of Surrey*
Corresponding author's email: s.richardson@manchester.ac.uk

Traumatic impact of cartilage within the joint and can lead to rapid chondrocyte cell death by excessive calcium influx and eventually matrix degradation, resulting in the development of post-traumatic osteoarthritis (PTOA). PTOA accounts for 5.6 million cases of osteoarthritis annually and costs $11.79 billion dollars annually. We have previously demonstrated that urocortin-1 has a protective effect on chondrocytes, preventing cell death following impact loading. Here we aimed to fabricate colloidal/liposomal nanoparticles for the delivery of urocortin-1 to joints to prevent chondrocyte death.
Colloidal (hyaluronic acid-chitosan) nanoparticles with urocortin-1 were loaded at 10-40% (w/w to CS). All particles showed a time-dependent increase in size. Chitosan-urocortin-1 complexation appeared unstable, compared with the addition of hyaluronic acid, suggesting urocortin-1-hyaluronic acid complexation. All PDI values remained below 0.25 over 15 days for up to 40% loading of urocortin-1. Liposomes (DMPC/cholesterol) were fabricated using a microfluidics approach (Dolomite, UK; flow rate ratios (FRR) 1:9-1:49; total flow rates (TFR) 250-750 µl/min). Larger FRRs (1:19, 1:49) displayed a smaller polydispersity index (PDI) with larger TFRs (500, 750 µl/min, $p<0.0001$). Zeta potential remained constant. Urocortin-1 (10% v/v, 100 µg/ml) loading resulted in increased size ($p<0.001$). Urocortin-1 mechanism of action was validated using the agonist Yoda1 on porcine articular chondrocytes. Intracellular Ca2+ showed decreased staining following supplementation with urocortin-1.
These results show the possibility of successful loading in biocompatible delivery mechanisms for sustained release of urocortin-1. These results detail the primary steps in producing a disease modifying OA drug (DMOAD) for use following cartilage injury in prevention of PTOA.

Keywords
Chondroprotection; Post-traumatic osteoarthritis

Abstract **#2283**

INCORPORATION OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES INTO 3D SCAFFOLDS FOR BONE TISSUE REGENERATION

Isabel Izquierdo-Barba[1,2], Manuel Estevez[1], Giorggia Montalbano[3], Clarissa Tomasina[4], Amadeo Franco-Bonatti[5], Montserrat Colilla[1,6], Blanca Gonzalez[1,2], Sonia Fiorilli[3], Lorenzo Moroni[4], Carmelo De María[5], Giovanni Vozzi[5], Chiara Vitale-Brovarone[3], María Vallet-Regí[1,2]

[1] *Universidad Complutense de Madrid*

[2] *CIBER-BBN*

[3] *Politecnico di Torino*

[4] *Maastricht University*

[5] *University of Pisa*

[6] *CIBER BBN*

Corresponding author's email: ibarba@ucm.es

Both versatility and biocompatibility of superparamagnetic iron oxide nanoparticles (SPIONs) make them ideal candidates to be used in a wide range of biomedical applications.[1,2] Herein, SPIONs have been synthesized, characterized and incorporated into different 3D scaffolds fabricated by either electrospun or Fused Deposition Modelling (FDM) techniques. For this purpose, stable and homogeneous SPIONs with a particle size of 12 nm were synthetized by thermal decomposition and transferred to aqueous medium via ligand exchange with dimercaptosuccinic acid obtaining stable colloidal suspensions.[3] Subsequently, the incorporation of the SPIONs into each type of 3D scaffold has been optimized. For electrospun scaffolds, the SPIONs were incorporated into type I collagen solutions resulting in a hybrid formulation suitable for electrospinning deposition. For FDM scaffolds the incorporation of SPIONs was performed by thermal Drop-on-Demand inkjet printing onto polymeric FDM scaffolds. This technique consists of ejecting picoliter droplets of liquid material through a nozzle thanks to a heating element that produces bubbles inside the solution [4]. Physicochemical characterization as well as in vitro stability assays of SPIONs-containing scaffolds have been conducted. Furthermore, their biological response in pre-osteoblastic MC3T3-E1 cells and human bone marrow derived mesenchymal stem cells (hBM-MSCs) has been evaluated to determine if the presence of SPIONs affects cell viability, adhesion and spreading. These findings confirm that both types of SPIONs-containing scaffolds constitute ideal platforms to explore magnetic mechano-stimulation to promote bone tissue regeneration. This research has been carried out within the framework of the GIOTTO project from the European Commission (GA 814410).

Keywords

superparamagnetic iron oxide nanoparticles; electrospun scaffolds; fused deposition modelling scaffolds

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Abstract **#2284**

MULTISCALE FRACTAL SHAPE CUES SUPPORT HIERARCHICAL ASSEMBLY AND MATURATION OF PODOCYTES VIA CURVATURE-INDUCED EXTRACELLULAR MATRIX PATTERNING

Anastasia Koroljp[1]

[1] *University of Toronto*

Corresponding author's email: anastasia.korolj@gmail.com

Nature efficiently self-organizes cells and tissues into complex fractal forms. Whether fractal patterning contributes functionally to maturation, and how cells sense and interpret such shape cues, is not well understood. Using kidney podocytes as a model system, bioinspired templating of glomerular histology was leveraged to design controlled fractal 2½ - D surfaces for cell culture. Microcurvature was associated with charge density gradients in space, found to direct extracellular matrix protein organization resulting in hierarchical assembly of cell structures and fractally-branching podocyte morphology in vitro, outlined with a novel fluorescent assaying technique. Shape simulation was uniquely associated with mature-like foot processes on an organized ECM. In applications of drug testing, coronavirus infection, and a cells-as-sensors approach to patient serum diagnostics, fractally stimulated cells were more responsive than flat cultures. Fractal frameworks may thus provide a functional role in podocyte maturation and could serve to advance other bioengineered systems.

Abstract **#2285**

NANOMATERIALS FOR BONE INFECTION MANAGEMENT

Isabel Izquierdo-Barba[1,2], Montserrat Colilla[1,2], Blanca Gonzalez[1,2], María Vallet-Regí[1,2]
[1] *Universidad Complutense de Madrid*
[2] *CIBER-BBN*
Corresponding author's email: ibarba@ucm.es

Biofilm-related infections associated to orthopaedic implants constitute one of most problematic chronic infection diseases with high morbidity, and high medical care expenses. The efforts are focused on the design of anti-infective biomaterials for both prevention and cure. The properties of these materials must be adapted to achieve anti-infective performance and biocompatibility, which permit a good integration of the implant with the surrounding tissue. Herein, the different strategies developed by our research group to engineer antibacterial nanomaterials will be described. In particular, the design of antimicrobial coatings, comprising innovative technological advances in surface modification; multifunctional 3D scaffolds with both antimicrobial and bone regeneration capabilities; and multifunctional hybrid nanomaterials as targeted therapies based on mesoporous silica nanoparticles, will be presented.

Keywords
antibacterial coatings; 3D scaffolds; mesoporous silica nanoparticles

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Abstract **#2286**

ACCELERATE BIOMEDICAL INNOVATIONS. TOGETHER.

Kurt Gielen$_P$[1]
[1] *Medace BV*
Corresponding author's email: kurt.gielen@medacespace.com

Too many promising medical innovations never reach the market or the patient. One of the hurdles to take is finding your way through the ever more stringent and difficult landscape of medical devices and cell therapy (GMP) legislation and technical validation.
Medace offers a full service work-learning environment where customers (being academics or entrepreneurs) are guided in how to set up and develop the technical dossier needed for clinical validation whilst producing their clinical grade product.
Medace works with an EN ISO 13485:2016 certified quality and service package available for our customers on a use by need basis.
Medace provides:
• Specialized infrastructure (e.g. ISO7 and 8, and Class C and B cleanrooms)
• Validated and specialized equipment
• Quality Management System
• Trained professionals for hands-on guidance and support during development and validation
• Dedicated in-house training programs

Keywords
Co-work space

Abstract **#2287**

3D-PRINTED IMPLANTS - WHAT ARE THE REQUIREMENTS FOR CLINICAL DATA UNDER THE EU MDR?

Matthias Fink[P][1]
[1] *TUV SUD America*
Corresponding author's email: matthias.fink@tuvsud.com

This presentation will highlight the requirements on clinical data for medical devices under the EU MDR with a focus on custom-made devices and 3D-printed implants. Summary of current interpretation of the EU MDR from a Notified Body's perspective based on the currently available MDCG guidance documents. The requirements for premarket clinical investigations and Post-market Clinical Follow-up (PMCF) studies will be addressed.

Keywords
Medical Device Regulation; Orthopedic Implants; Notified Body

Abstract **#2288**

PATIENT MATCHED WRIST IMPLANTS MADE BY RAPID TOOLING TECHNOLOGIES

Aylvin A Dias$_P$[1]
[1] *DSM Biomedical*
Corresponding author's email: aylvin.dias@dsm.com

Patient Matched Wrist Implants Made by Rapid Tooling Technologies
Aylvin A. Dias1, P. Schormans2, H. Keul1, J. Mommers1 J. Gruisen2, M. Kozlowska1, I. Punt2 M. Beerens3,
1. DSM Biomedical 2. Maastricht University Medical Centre, 3. Xilloc Medical Int B.V.

Wrist osteoarthritis is a result of mechanical abnormalities resulting in joint destruction occurring in the wrist. These abnormalities include degeneration of cartilage and hypertrophic bone changes, which can lead to pain, swelling and loss of function, occupational disability with a significant socio -economic impact.
25% of injuries in the Netherlands presented in the emergency departments in the Netherlands are hand and wrist injuries. Post-traumatic osteoarthritis of the wrist, especially the radiocarpal joint, is a possible consequence of wrist trauma and can lead to severe functional impairment and pain.
Clinical results of available surgical techniques for treatment of post-traumatic radiocarpal osteo-arthritis are disappointing due to limited mechanical properties and poor fit of the available materials. A possible solution might be the insertion of a patient specific interpositional arthroplasty in the affected joint with materials with improved ergonomic and mechanical properties.
In this study, we will describe the clinical, regulatory and technical challenges (3D printing versus rapid tooling) that were addressed in preparation of patient matched wrist implants for a first in human clinical trial planned in 2022.

Acknowledgments: This research was performed under the framework of Chemelot InSciTe

Keywords
osteoarthritis; arthroplasty; trauma

References
R. Van Leerdam et. al. Incidence and treatment of hand and wrist injuries in Dutch emergency departments Eur. J. Trauma Emerg. S. July 2021 DOI: 10.1007/s00068-021-01732-x

Abstract **#2289**

ENGINEERED SCAFFOLDS FOR THE DELIVERY OF GENE THERAPEUTICS FOR ENHANCED TISSUE REPAIR

Fergal J O'Brien[P][1]
[1] *Royal College of Surgeons in Ireland*
Corresponding author's email: fjobrien@rcsi.ie

The COVID-19 pandemic has shown how revolutionary treatments based on gene therapeutics has helped overcome a once-in-a-century pandemic and has given new momentum to gene therapy research for a myriad of applications. The field of regenerative medicine is well placed to be a beneficiary whereby, for example, gene therapy might be a valuable tool to avoid the limitations of local delivery of growth factors. While non-viral vectors are typically inefficient at transfecting cells, our group have had significant success in this area using a scaffold-mediated gene therapy approach for regenerative applications[1, 2]. These gene activated scaffold platforms not only act as a template for cell infiltration and tissue formation, but also can be engineered to direct autologous host cells to take up specific genes and then produce therapeutic proteins in a sustained but eventually transient fashion. Similarly, we have demonstrated how scaffold-mediated delivery of siRNAs[3] and miRNA[4, 5] can be used to silence specific genes associated with reduced repair or pathological states. This presentation will provide an overview of ongoing research in our lab in this area with a particular focus on gene-activated biomaterials for promoting bone, cartilage, nerve and wound repair. Focus will also be placed on advances we are making in using 3D printing of gene activated bioinks to produce next generation medical devices for tissue repair.

ACKNOWLEDGEMENTS: European Research Council Advanced Grant, ReCaP (agreement n° 788753)

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Abstract **#2290**

PROOF-OF-CONCEPT EVALUATION OF CANINE MULTIPOTENT ADULT PROGENITOR CELLS (ML-223) IN CANINE CARTILAGE DISEASE MODELS

Annelies Bogaerts[P][1], Mark Hurtig[2], Saartje Walbers[1], Aline Visser[1], David Craeye[1], Sylvester G Price[3]
[1] *ReGenesys*
[2] *University of Guelph*
[3] *Boehringer Ingelheim Vetmedica GmbH Research*
Corresponding author's email: abogaerts@regenesys.eu

The potential role of mesenchymal stem cells (MSC) to impact osteoarthritis (OA) mechanisms has been established in preclinical models and in pilot clinical trials. Multipotent Adult Progenitor Cells (MAPC®) represent a unique stem cell population with regenerative properties that differ from MSCs by morphology, gene expression profile, proliferative capacity, and functions. This study was conducted to test the effect of canine MAPC (cMAPC; ML-223) on cartilage disruption and inflammation in the groove model (GM) and in the medial meniscal release model (MMRM), 42 and 70 days after cell therapy initiation. These two cartilage disease models of OA were evaluated because each model represents different pathologic mechanisms of the natural disease.
cMAPC prevented disease progression and promoted cartilage repair in the GM but not in the MMRM. cMAPC protected against index cartilage lesion enlargement, conferred protection in adjacent and apposing joint surfaces, and subdued subchondral bone sclerosis. This suggests cMAPC have potential clinical applications for cartilage repair in: (1) aging where slowly progressive OA arises from superficial cartilage wear lines and small erosions that coalesce to create global joint degeneration and (2) acute mechanical cartilage injury (bone bruise or bone marrow edema).
The gene expression data demonstrate that cMAPC treatment impacted immunological components in both OA models. Treated dogs have an altered immune cell phenotype involving Tregs. These data also demonstrate the importance of understanding the time course of immunologic perturbations induced by the GM and MMRM and optimizing treatment time points when designing investigation of regenerative therapeutics.

Abstract **#2291**

MOLECULAR ASPECTS OF CELLULAR SENESCENCE IN MSCS AND ITS IMPACTS ON DIFFERENTIATION

Roberto Narcisi[1]
[1] *Department of Orthopaedics and Sports Medicine, Erasmus MC, University Medical Center, 3015 CN Rotterdam, the Netherlands*
Corresponding author's email: r.narcisi@erasmusmc.nl

Cellular senescence is an irreversible state in which cells stop proliferating, while remaining metabolically active, showing a specific senescence-associated secretory phenotype (SASP). Extensive proliferation is one of the factors leading to cellular senescence. In mesenchymal stem/stromal cells (MSCs), progenitor cells present in several body tissues, senescence can be also observed both in vitro and in vivo. However, the molecular aspects of cellular senescence in MSCs are still poorly understood, as well as the possible impact of senescence on the differentiation capacity of these cells.
Previously we observed that activation of the WNT/β-catenin pathway sustains MSC expansion, while maintaining their multilineage differentiation capacity [1-2]. Interestingly, later we observed that the WNT/β-catenin pathway is sustaining expansion not by acting as direct pro-proliferative signal, but by blocking senescence instead, via repressing the SASP-related factors (under review). Further characterization showed that the transcription factor TWIST1, a WNT/β-catenin target, appeared as one of the main responsible for the effect observed on senescence and on differentiation capacity of MSCs [3-4]. Additional investigations highlighted the role of senescence as negative regulator of MSC differentiation, with a greater impact on reducing chondrogenesis capacity rather than adipogenesis or osteogenesis (under review). Finally, we also found that senescent MSCs have an altered capacity to respond to TGFβ, the main pro-chondrogenic factor of MSCs (under review).
Overall, our data indicate that the effect of senescence on MSCs depends on the differentiation stage of the cells, highlighting the role of WNT and TWIST1 as potential targets for blocking senescence in MSCs.

Keywords
Senescence; Differentiation; Cellular metabolism

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Abstract **#2292**

ROLE OF VASCULAR SMOOTH MUSCLE CELLS IN VASCULAR REMODELING

Leon Schurgers[P][1]
[1] *Maastricht University*
Corresponding author's email: l.schurgers@maastrichtuniversity.nl

Vascular calcification is a feature of vascular remodeling and was regarded as a bystander in cardiovascular disease. Vascular calcification was considered to be the passive chemical nucleation of calcium and phosphate on cellular debris and therefore the end-stage of atherosclerosis. This view has changed over the last two decades with cells and proteins involved in the vascular calcification homeostasis and vascular calcification being an independent risk factor for cardiovascular disease. Vascular smooth muscle cells (SMCs) are key cells in vascular remodeling, and phenotypic switching has been shown to be involved in vascular calcification.
SMCs, which build the arterial wall, show phenotypic plasticity. In physiological conditions they exist as contractile cells, regulating vascular tone. In response to stress and/ or injury SMCs lose expression of contractility-related proteins and proliferate, migrate and secrete extracellular matrix-related proteins in order to repair the damaged vessel. Dysregulated SMC phenotype switching lies at the heart of many vascular pathologies, such as atherosclerosis, aortic aneurysms, remodeling of hypertensive blood vessels and vascular calcification. The molecular determinants of phenotype switching are not fully understood.
To unravel these mechanisms, we employ a molecular biological approach, using primary patient derived SMCs and iPSC derived iSMCs. iPSCs allow us to generate vascular smooth muscle cells specific from a series of loci of the cardiovascular system. This approach will help us unraveling why in the clinic certain vascular sites exhibit a higher propensity of calcifications.

Abstract **#2293**

VIRTUAL MODELS FOR HUMAN DEVELOPMENTAL TOXICOLOGY DRIVEN BY NEW APPROACH METHODOLOGIES

Aldert Piersma[P][1], Harm Heusinkveld[1], Yvonne Staal[1], George Daston[2], Richard Spencer[3], Thomas Knudsen[3]
[1] *RIVM, Bilthoven, Netherlands*
[2] *P&G, Cincinatti OH, USA*
[3] *USEPA, Raleigh NC, USA*
Corresponding author's email: aldert.piersma@rivm.nl

Quantitative in silico modeling of network dynamics is an important goal toward predicting toxicological hazard from chemical-biological interaction. Systems toxicology mapping of network structure-function would reveal threshold events at the toxicological tipping point between adaptive and adverse biology. These events can be tested in dedicated new approach methodologies (NAMs) focusing on the molecular and/or cellular scale. Concentration-dependent compound-induced effects from targeted in vitro studies fed into the network model provide toxicity prediction at the level of the intact organism. These systems models utilize the extensive knowledge that exists on biological control of morphogenesis and the conservation of key biological pathways and processes enabling integration of data on among diverse species, . Current efforts towards in silico modeling of specific morphogenetic processes include, for example, neural tube closure. In a CEFIC-LRI sponsored project we described the biological pathway network underlying vertebrate neural tube closure, using a text mining tool applied to the open scientific literature. This network is the input for developing a 3-dimensional computational model for in silico testing of compound effects on neural tube closure. The principal idea is that by adapting parameter settings in the in silico model based on effects on gene expression observed in relevant in vitro cell assays, their consequences at the higher integration level of neural tube closure can be predicted. In due course these models are expected to revolutionize toxicity testing, enhancing human relevance based on enhanced insight into mechanisms of toxicity. Does not reflect EPA policy.

Abstract **#2295**

STEM CELL DERIVED KIDNEY TISSUE, AND ITS CHALLENGES FOR CLINICAL TRANSLATION

Ton J. Rabelink[P][1]
[1] *LUMC*
Corresponding author's email: t.rabelink@lumc.nl

The field of regenerative medicine aims to engineer, replace, or regenerate cells, organs or tissues in order to restore function after damage or loss. It has seen tremendous growth with the introduction of human induced pluripotent stem cells (hiPSC) Human adult kidneys filter the blood and are important for maintaining homeostasis by regulating electrolyte concentration, blood pressure and secreting hormones. The functioning units of the kidney are called nephrons and each kidney has on average 1 million nephrons. Due to chronic kidney disease, progressive loss of renal function can result in the need for renal replacement therapy. While dialysis is costly, time consuming, and only partially restores kidney function, transplantation is dependent on the availability of donor organs, and patients need immunosuppressive drugs and have the risk for acute or chronic rejection. Therefore, generation of bioengineered iPSC derived kidney tissue to replace kidney function is a field of interest. Knowledge on nephron progenitors obtained by studying the developing embryo in mice, resulted in advanced protocols for the generation of these structures, organoids in vitro. Kidney organoids show a remarkable level of complexity containing multiple cell types and upon transplantation these structures become vascularized and further mature. While these organoids are interesting candidates for kidney replacement tissue, there are still major hurdles to be overcome before they can be clinically applied. In this talk these challenges will be systematically addressed.

Abstract **#2296**

HYALURONAN HYDROGELS ATTENUATE THE IMMUNE RESPONSE IN THE CENTRAL NERVOUS SYSTEM

Molly S. Shoichet[P][1], Michael J. Cooke[1], Brian G. Ballios[1], Nikolaos Mitrousis[1], Dimpy Gupta[1], Catherine E. Kang[1], Tobias Fuehrmann[1], Samantha L. Payne[1], Jaclyn M. Obermeyer[1], Anup Tuladhar[1]
[1] *University of Toronto*
Corresponding author's email: molly.shoichet@utoronto.ca

Regeneration in the central nervous system remains a significant challenge, complicated further by that complexity of the system itself. To promote regeneration, we have been investigating strategies to overcome key challenges associated with survival and integration of cell therapy.

We found that a hyaluronan-based hydrogel comprised of hyaluronan and methyl cellulose (HAMC) attenuates the inflammatory response after traumatic injury in the spinal cord, brain and retina at the back of the eye [1]. To further build on this positive effect, we have investigated both exogenous cell transplantation and endogenous cell stimulation strategies using the HAMC hydrogel.

For example, in a rodent model of stroke, we found that the delivery of HAMC promoted tissue repair itself that was enhanced with either the delivery of neural stem cells [2] or brain-derived neurotrophic factor [3].

Similarly, in a rodent model of blindness, we found that HAMC enhanced the survival of retinal stem cell-derived photoreceptors via a CD44-mediated mechanism [4]; and that the co-delivery of photoreceptors with hESC-derived retinal pigmented epithelium further enhanced functional vision repair [5]. Interestingly, even in vitro, hyaluronan-based hydrogels improved photoreceptor cell survival [6].

The positive effects of the hydrogel itself and its combination with biologics and/or cells will be described.

Keywords
Blindness; Hydrogels; Stroke

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Abstract **#2297**

MACHINE LEARNING AND MATHEMATICAL MODELS FROM MULTI-OMICS DATA FOR PERSONALIZED MEDICINE

Julio Saez-Rodriguez[P][1]
[1] *University Hospital Heidelberg*
Corresponding author's email: admin.saez@uni-heidelberg.de

Modern technologies allow us to profile in high detail biological and medical samples at fast decreasing costs. New technologies are opening new data modalities, including to measure at the single-cell level and with spatial resolution. Computational models, in particular those built with machine learning, are expected to help us to extract insight form these data. Using biological knowledge to aid machine learning can significantly improve the results. Towards this end, we have developed a number of tools that range from a meta-resource of biological knowledge to methods to infer pathway and transcription factor activities from gene expression and subsequently infer causal paths among them. Furthermore, and to complement a large-scale basal profiling of samples, we have develop approaches to build dynamic logic models of molecular networks and how they response to perturbations such as drug treatment. I will illustrate their utility in cases of biomedical relevance and show how they improve our understanding of molecular processes, identify biomarkers, and point at novel therapeutic opportunities.

Abstract **#2298**

INNOVATION IN REGENERATIVE MEDICINE: PERSPECTIVES FROM A LARGE MEDICAL DEVICE COMPANY

Markus Reiterer[P][1]
[1] *Medtronic, PLC*
Corresponding author's email: markus.w.reiterer@medtronic.com

Over the last 70 years medical technology has positively impacted millions of lives every year. While, devices like spinal implants, cardiac stents, and pacemakers have restored function and improved health, healing is hardly ever achieved. Regenerative medicine provides a promise of cure that is achieved by administering bioactive materials, biologics, cell therapies or tissue engineered products. Although the regenerative medicine market has been estimated to be in the $10B by the mid-2020s, only very few successful products exist today. The problems are manyfold, from failed clinical trials to difficulties with cost-effective and quality-controlled high volume manufacturing, to regulatory uncertainty that slow down investments. A large medical device company like Medtronic needs to be super vigilant due to our size an ability to impact many patients. Understanding underlying technologies of manufacturing in regenerative medicine, mechanisms of action of the proposed therapies and leveraging technological and market adjacencies will contribute to successful implementation.

Keywords
Industry; Innovation

Abstract **#2299**

TYMPANIC MEMBRANE SCAFFOLDS AIDED BY CHITIN NANOFIBRILS TO MODULATE INFLAMMATORY AND IMMUNE RESPONSE

Bahareh Azimi$_P$[1], Lorenzo Zavagna[2], Claudio Ricci[1], Alessandra Fusco[3], Mario Milazzo[4], Shivesh Anand[5], Giovanna Donnarumma[3], Carlos Mota[5], Serena Danti[1]

[1] *University of Pisa*

[2] *Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM)*

[3] *University of Campania "Luigi Vanvitelli"*

[4] *Scuola Superiore Sant'Anna*

[5] *Maastricht University*

Corresponding author's email: serena.danti@unipi.it

Chitin nanofibrils (CNs) are a bio-based nanomaterial obtained from the purification of chitin. Their nanometric size and high crystallinity enable anti-inflammatory, cicatrizing, and anti-ageing activity [1]. We investigate the possible use of CNs to modulate the inflammatory and immune response occurring upon tympanic membrane (TM) scaffold implantation as a consequence of infections, in order to avoid device rejection or accelerated degradation [2].

In this study, two different types of CN integration in TM scaffolds were investigated. A nanocomposite was produced by melt extrusion of CN/polyethylene glycol (PEG) pre-composite (50/50 weight ratio) and poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT), achieving a final CN concentration of 2% (w/w%). The composite was electrospun into fibrous scaffolds, which were coated by CNs from crustacean or fungal sources via electrospray. The degradation behaviour of the scaffolds was investigated during 4 months at 37°C in an otitis-simulating fluid.

In vitro tests were performed to assess cytocompatibility and immune response. Human mesenchymal stem cells (hMSCs) and human dermal keratinocytes (HaCaT cells) were cultured as models of connective and epithelial tissues, respectively. HMSCs were able to colonize the scaffolds and produce collagen type I. HaCaT cells in contact with the CN-coated scaffolds revealed a marked downregulation of the proinflammatory cytokines. CN-coated PEOT/PBT/(CN/PEG 50:50) scaffolds showed a significant indirect antimicrobial activity. The nanocomposite was biostable until the endpoint.

Acknowledgements: This study was funded by 4NanoEARDRM project (EuroNanoMed III H2020), Netherlands Organization for Scientific Research (NWO, G.A. OND1365231), and the Italian Ministry of Education, University and Research (MIUR, G.A: B56H18000140001).

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Abstract **#2300**

ELECTROSPRAYED SHRIMP AND MUSHROOM CHITIN NANOFIBRILS FOR SKIN CONTACT APPLICATION

Lorenzo Zavagna[P][1], Bahareh Azimi[2], Claudio Ricci[2], Maria-Beatrice Coltelli[2], Andrea Lazzeri[2], Patrizia Cinelli[1]
1 Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM)
2 University of Pisa
Corresponding author's email: serena.danti@unipi.it

Materials recovered from biowaste can still be used to produce effective skincare product with a reduced environmental impact. Chitin is a natural polysaccharide found in the shells of crustaceans, cuticles of insects, and fungal cell walls. In nanofibrillar form, chitin loses its pro-inflammatory and allergenic character, and proficiently interacts with many cellular compounds in biological tissues [1].
This research investigated electrospray as a method for surface functionalization of cellulose tissues with chitin nanofibrils (CNs) using two different sources (from fungi and crustaceans) and different solvent systems to obtain a biobased, skin compatible product with enhanced anti-inflammatory activity [2].
The surface of cellulose tissues was uniformly decorated with electrosprayed CNs. The effects of different solvents (i.e., water-based and organic) were investigated to assess the surface nanostructure via scanning electron microscopy (SEM) and the final skin compatibility using human dermal keratinocytes (HaCaT cells). Biological analysis revealed that all treated samples were suitable for skin applications. These results indicate that the use of solvents did not affect the final cytocompatibility due to their effective evaporation during the electrospray process. Such treatments did not affect the characteristics of cellulose; in addition, they showed promising in vitro anti-inflammatory and indirect antimicrobial activity. The results prove that electrospray could represent a green method for surface modification of sustainable and biofunctional skincare products.

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Abstract **#2301**

ELASTIN LIKE RECOMBINAMERS, VERSATILE TOOLS FOR CONTROLLED ANGIOGENESIS IN CARDIAC TISSUE ENGINEERING

Israel Gonzalez de Torre[P][1,2]
[1] *BIOFORGE, Universidad de Valladolid, España*
[2] *Center for Biomedical Research in the Network in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.*
Corresponding author's email: igonzalez@bioforge.uva.es

Acute myocardial infraction (AMI), traditionally defined as a decrease of stoppage of blood flow to a portion of the cardiac muscle leading to a necrotic tissue. It is usually result as a consequence of a discrepancy between the amount of oxygen that the cardiac muscle requires and the supply that effectively receives. This discrepancy may lead to a myocardial damage, usually in the left ventricle. As in any other muscular tissue, the subsequent ischemia produces a necrotic wound with reduction of the capacity of the affected area to develop its normal function. Elastin like recombinamers (ELRs) are synthetic recombinantly produced polypeptides that mimic natural elastin, an essential ECM component. These polypeptides can be scaled up and are amenable to mechanical and bioactive customization. Several strategies to obtain angiogenesis and a better scaffold colonization searching for a functional tissue reparation have been explored with ELRs along the last five years. From evaluate the porosity to the inclusion of different bioactivities that open the possibility to modulate and control the level of angiogenesis, program controlled spatial-temporal degradations ELRs are playing an important role in cardiac tissue engineering applications, reaching preclinical tests in animals with excellent outcomes.

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Abstract **#2302**

INNER EAR ORGANOIDS: A NEW TOOL FOR UNDERSTANDING AND TREATING DEAFNESS

Brigitte Malgrange[P][1]
[1] *University of LIEGE - GIGA stem cells*
Corresponding author's email: bmalgrange@uliege.be

Sensorineural hearing impairment is a common pathology affecting more than 16% of the population worldwide. It depends on a specialized sensory organ called the cochlea containing auditory hair cells and the spiral ganglion neurons. To date, treatment options are limited to hearing aids and cochlear implants. Therefore, developing therapies to replace missing cells is essential. The discovery of human-induced pluripotent stem cells (hiPSCs) has allowed the derivation of patient-specific stem cells that can differentiate into otic sensorineural cell derivatives. This promising tool will facilitate the study of disease mechanisms, bioengineering, and stem cell-based cell replacement therapies either as individual cell types or as organoid three-dimensional cultures. Here, we will highlight significant accomplishments and challenges associated with hiPSC-derived otic cells as a new tool to understand pathogenesis and identify novel therapies.

Abstract **#2303**

IMMUNOGENIC REACTIONS TO BIOMATERIALS

Martijn van Griensven[P][1]
[1] *Department cBITE, MERLN Institute, Maastricht University*
Corresponding author's email: m.vangriensven@maastrichtuniversity.nl

Biomaterials are implanted in organisms in an array of different tissues and for the purpose of several applications. This will always lead to local tissue injury and an interaction of the biomaterial with the surrounding tissues. Moreover, vascular injury is also present. This results in a contact of the biomaterial with the blood coming from the disrupted vessels. Both in the tissues as well as in the blood, immunologic active cells are present. Depending on the material, a minimal or stronger immunological reaction will occur. This talk deals with these immunogenic reactions to implanted biomaterials based on different sources such as natural, allogeneic, xenogeneic, or synthetic. The immunogenic reaction is part of the biocompatible properties of a biomaterial. Biocompatibility is defined as 'the ability of a material to perform with an appropriate host response in a specific application'. The host response is mainly constituted by the host immune reaction. When the immune reaction towards the biomaterial is too strong, that is an inappropriate host response, the material may be rejected. On the other hand, a minimal immune reaction is necessary for the biomaterial to be integrated in the organism. When no interaction with the biomaterial takes place, it will have no immediate interaction with the tissue. Thereby, most of its function will get lost. Thus, the intensity combined with the duration of the inflammatory reaction is an important aspect of biocompatibility and thus for regenerative success.

Keywords
immune response; biocompatibility; biomaterial

Abstract **#2304**

BOOSTING ENDOGENOUS REPAIR OF CARTILAGE: FOCUS ON THE MOLECULAR IDENTITY OF THE ARTICULAR CHONDROCYTE

Rik Lories[P][1]

[1] *KU Leuven*

Corresponding author's email: rik.lories@kuleuven.be

Osteoarthritis (OA), the most common chronic joint disease, is characterized by progressive damage to the articular cartilage, increased joint-associated bone remodeling and varying degrees of inflammation. OA leads to loss of joint function and is one of the leading causes of disability worldwide. Current therapy is limited to symptom relief and in severe cases joint replacement surgery, while interventions that arrest or reverse disease progression are lacking. The onset of clinical symptoms (pain, stiffness and impaired mobility) is preceded by molecular and cellular changes in the joint that result in loss of homeostasis. Different factors can initiate such a process and include trauma, aging and mechanical stress, all in interaction with the genetic predisposition of the individual patient. The articular chondrocytes, the unique cells of the articular cartilage, are embedded in a self-produced extracellular matrix of collagen fibers and proteoglycans. In OA, these chondrocytes lose their characteristic molecular identity, and often acquire a hypertrophy-like phenotype. The switch from the quiescent and stable molecular phenotype of articular chondrocytes to the metabolically more active hypertrophic phenotype observed in OA is associated with hyper-activation of the Wnt Wnt/β-catenin signaling pathway. Thus, unravelling regulatory mechanisms that control Wnt signaling and Wnt-mediated chondrocyte hypertrophy may lead to identification of effective therapeutic targets

Abstract **#2305**

UNIQUE SUPPLY CHAIN SOLUTION FOR STEM CELL TREATMENT WITH SHORT SHELF-LIFE.

Sebastian Wehle[P][1]

[1] *Takeda Pharmaceutical Int. AG, Zürich, Switzerland*

Corresponding author's email: sebastian.wehle@takeda.com

Darvadstrocel is a live human mesenchymal stem cell treatment indicated for complex perianal fistula(s) in adult patients with non-active/mildly active luminal Crohn's disease, when fistula(s) have shown an inadequate response to at least one conventional or biologic therapy. By the nature of a living cell formulation the shelf-life of the finished product is 72 hrs. which requires a unique supply chain and innovative engineering solutions.

Since the program ideation phase, the Darvastrocel team has been envisioning a unique bespoke end-to-end supply chain solution spanning over donor´s tissue extraction up to drug product administration to patients within the short product shelf-life of 72 hrs.

The unique features relate to:

• Fit for purpose combination of Takeda´s resource planning (ERP) system and the logistic service provider led by a control tower function. The seamless connectivity of key information system with IoT cloud based communication tools at the hospital level unlocks capabilities for order placement, order status monitoring, on-time delivery tracking and coordination across multiple stakeholders.

• Dedicated in-house manufacturing network able to parametric release the finished product precise on the aligned hour for pick up by the courier service provider.

• Supply network "horizontal" patient oriented along the end to end supply chain rather than "vertical" by site functions.

• High automated degree to be nearly paper free to support parametric release on the hour.

• Rapid analytical methods installed to accomplish short shelf-life of the product.

Keywords

End to end Supply Chain solution for short shelf-life product; Parametric release on-time; Mesenchymal Stem Cell Therapy

Abstract **#2306**

CELLULAR ORGANELLE-MIMICKING SUPRAMOLECULAR PEPTIDE NANOSTRUCTURES FOR MEDICINAL APPLICATIONS

Tanja Weil[1], David Y.W. Ng[1], Christopher V. Synatschke[1]
[1] *Max Planck Institute for Polymer Research*
Corresponding author's email: weil@mpip-mainz.mpg.de

The extracellular matrix (EM) forms a complex, supportive environment for cells providing mechanical and biochemical cues, and enabling cells to communicate. [1] Evolution has created these unique heterogeneous matrices with inner structure and various functional entities that control e.g. pathogen binding and stimulate cell growth and division. We focus on the synthesis of peptide-polymer biomaterials that can serve as synthetic EM-mimicking environments by forming 3D fibril networks [2] or soft polymers with inner fibril structure [3]. Rational optimization of monomer sequences and fibril morphologies facilitated dynamic supramolecular networks supporting neuronal cells growth in vitro and vivo [4] as well as pathogen binding [5]. Responsiveness towards various stimuli such as pH changes, light and reactive oxygen species was implemented into the biopolymer sequences. In this way, materials properties were optimized towards enhanced bioactivity and customized for their respective microenvironments for e.g. local drug delivery [6], which offers new avenues for tissue engineering.

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Abstract **#2307**

THE INTERPLAY BETWEEN HEMOSTASIS AND IMMUNE RESPONSE IN BIOMATERIAL DEVELOPMENT FOR OSTEOGENESIS

Corresponding author's email: yin.xiao@qut.edu.au

Treatment of large bone defects remains a clinical challenge, especially in the sizeable segmental defect, which usually results in bone non-union. In such a clinical scenario, bone substitutes are often applied. The gold standard bone substitute is still the autologous bone graft and is difficult to be replaced by synthetic biomaterials, suggesting that strategies should be made to improve the material for functional bone regeneration. Recent studies have revealed that hematoma, the first tissue structure formed at the bone injury site, plays an indispensable role in bone healing. Hematoma consists of fibrin clot, inflamed immune cells and tissue progenitor cells, which not only bridges the bone defect, but also provides a microenvironment for the interplay between hemostasis and immune systems. Previous studies have found that an ideal fibrin structure with proper fiber thickness and density was found to benefit progenitor cell infiltration and differentiation, and biomaterial implantation could affect bone healing by altering fibrin structure. Meanwhile, immunoregulation plays an indispensable role in bone healing, especially, materials inducing a shift from inflammatory to anti-inflammatory phenotypes in immune cells showed enhanced osteoinductivity. More importantly, the interaction between the hemostasis and immune systems should play a vital part in bone regeneration, by determining both the fibrin structure and the bone healing microenvironment. The hemostasis and immune systems are entangled to mediate the balance between coagulation and anti-coagulation, as well as to restrain the inflammation at a certain level. Coagulants triggered inflammation could in-turn facilitate coagulation cascades, which forming a positive feed-back to amplify both processes. On the other hand, anti-coagulants neutralize coagulation and inhibit inflammation, thereby control the coagulation and inflammation to prevent thrombosis. The balance between coagulation—inflammation and anti-coagulation—anti-inflammation plays a determinant role in not only fibrin structure but also the fibrinolysis process, during which the inflammation could be gradually “quenched” hence benefiting osteogenesis, and resulting in fibrin fragments favoring angiogenesis, thereby generating an ideal microenvironment for the following bone regeneration. Currently there are limited biomaterial studies targeting the bone-healing hematoma, especially the hemostasis-immune interplay. Thereby, this presentation summarizes the current materials for hemostasis and immunomodulation, the critical role of hemostasis-immune correlation in bone regeneration, and further propose the potential strategies to develop materials with the capacities to generate an ideal bone healing hematoma, by modulating the hemostasis-immune interplay to maintain the balance between coagulation—inflammation for osteogenesis.

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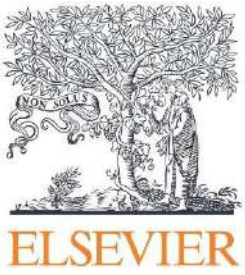

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