Cell-Matrix Interaction and Mechanobiology in Regenerative Medicine

Brno, 10th-11th June 2021

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Virtual TERMIS workshop: "Cell-Matrix Interaction and Mechanobiology in Regenerative Medicine"

For the first time TERMIS goes to Czechia!

The International Clinical Research Center of St. Anne University Hospital, Brno, and Masaryk University are pleased to announce the **TERMIS workshop**: "**Cell-Matrix Interaction and Mechanobiology in Regenerative Medicine**" taking place on June 10 – 11 2021 in Brno, Czech Republic, the birthplace of modern genetics.

The decision to run the workshop from remote was not easy, because it took away from participant and organizers the funny part of the scientific meetings: gathering, networking, unloading.

Nonetheless, the scientific value of the meeting remains, with a mix of outstanding international speakers and young scientists talks.

TERMIS workshop "Cell-Matrix Interaction and Mechanobiology in Regenerative Medicine" will bring together students and researchers from a broad range of disciplines with a common focus on mechanobiology and cell-matrix biology applied to organ development, regeneration, and tissue engineering.

Although at this time we will not be able to host you in person in the city where Gregor Mendel made his discoveries, it will be a pleasure to see known and new faces from remote.

Looking forward to meeting you!

On behalf of the organizing committee:

Giancarlo Forte
Vladimír Vinarský
Jorge Oliver-De La Cruz
Soraia Fernandes
Stefania Pagliari
Tomáš Bárta
Dáša Bohačiaková
Heinz Redl
Johannes Grillari

WORKSHOP PROGRAM

Join the Webinar via Zoom platform: https://zoom.us/webinar/register/WN_jCC8Nv8yTzS4COvrflT_8Q

Webinar ID: 966 2619 3006

THURSDAY JUNE 10, 2021

9.00 – 9.10 **Welcome session: Giancarlo Forte**, FNUSA-ICRC, Czech Republic

SESSION 1: CELL MECHANICS IN DEVELOPMENT AND REGENERATION

Discussion chairs: Heinz Redl (LBI, Wien) and **Vladimír Vinarský** (FNUSA-ICRC, Brno)

9:10 – 9:55 **Pere Roca-Cusachs**, IBEC, Spain
Transducing - and shielding - mechanical signals from integrins to the nucleus

9:55 – 10:25 **Diana S. Nascimento**, ICBAS/i3S/INEB, Portugal
The Yin and Yang of the cardiac extracellular matrix: from regeneration during development to repair in adulthood

10:25 – 10:55 **Giancarlo Forte**, FNUSA-ICRC, Czech Republic
New paradigms in pathological mechanosensing

10:55 – 11:10 COFFEE BREAK

11:10 – 11:40 **Guido Caluori,** LIRYC INSERM U1045, France
Methods and tools to investigate cell mechanobiology

11:40 – 12:10 **Paolo Maiuri**, IFOM, Italy
The nucleus: Polarity and Volume

12:10 – 12:55 Selected talks from abstracts (10 min + 5 min Q&A)

12:10 – 12:25 **Lina Papadimitriou**, IESL FORTH, Greece
Influence of topography of micro-patterned Si surfaces on PC12 neural cell differentiation: implication of mechanotransduction pathways
12:25 – 12:40 **Gloria Garoffolo,** CCM, Italy
Convergence of Hippo/TGF-β pathways in mechanical activation of vein aorto-coronary bypass failure
12:40 – 12:55 **Fabiana Martino,** FNUSA-ICRC, Czech Republic
hnRNPC mediates the mechanoregulation of mRNA homeostasis in heart failure

12:55 – 14:00 LUNCH BREAK

SESSION 2: MATRIX SENSING IN DEVELOPMENT AND REGENERATION

Discussion Chairs: Giancarlo Forte (FNUSA-ICRC) and **Wolfgang Holnthoner** (LBI)

14:00 –14:30 **Oleg Lunov**, FZU, Czech Republic
In vitro Models for Evaluation of Drug-Induced Liver Injury: Role of Physical Factors

14:30 –15:00 **Valerio Izzi**, University of Oulu, Finland
System-level models of the expression and mutations/alterations of the tumor matrisome

15:00 – 15:15 COFFEE BREAK

15:15 – 16:00 **Sirio Dupont**, University of Padova, Italy
ECM mechanotransduction licences an antioxidant response by regulating mitochondrial morphology and NRF2 activity

16:00 – 16:45 Selected talks from abstracts (10 min + 5 min Q&A)

16:00 – 16:15 **Andreas Traweger,** PMU, Germany
The Matricellular Protein SPARC Modulates Load-sensing in Tendons
16:15 – 16:30 **Eleftheria Babaliari**, IESL FORTH, Greece
Study of the combined effect of shear stress and laser-patterned topography on Schwann cells' behavior
16:30 – 16:45 **Jorge Oliver-De La Cruz**, FNUSA-ICRC, Czech Republic
Nucleoskeletal organization is influenced by YAP-dependent extracellular matrix rearrangement

16:45 – 18:00 Rapid-fire poster presentations (15 min each video + 10 min Q&A)
16.45-17.00 Poster session: Cell, tissue dynamics and patterning
17-10-17.25 Poster session: Materials for regeneration
17.35-17.50 Poster session: Disease Modelling

Additional information about the posters presented in each session can be found in pages 7-8.

FRIDAY JUNE 11, 2021

SESSION 3: MATRIX MIMICRY TO FOSTER REGENERATION

Discussion Chairs: Sirio Dupont (University of Padova) and **Soraia Fernandes** (FNUSA-ICRC)

9:00 –9:45 **Carl-Philipp Heisenberg**, IST, Austria
A mechanochemical gradient controlling robust cell internalization during zebrafish gastrulation

9:45 – 10:15 **Michael Monaghan**, TCD, Ireland
Tailoring Electroconductive Biomaterial Patches and Scaffolds to Match the Mechanical Anisotropy of Organ-specific

10:15 – 10:45 **Serena Zacchigna**, ICGEB, Italy
In vivo secretome screening identifies emid2 anti-invasive properties through modulation of tumor microenvironment

10:45 – 11:00 COFFEE BREAK

11:00 – 11:30 **Annalisa Tirella**, University of Manchester, UK)
Advanced materials to mimic biophysical properties of tissue ECM in 3D

11:30 – 12:00 **Chiara Arrigoni** (Ente Ospedaliero Cantonale, Switzerland)
3D human microvasculature on a chip identifies novel therapeutic targets challenging cancer cell extravasation

12:10 – 12:15 Selected talk from abstracts (10 min + 5 min Q&A)

12:00 – 12:15 **Cian O'Connor,** RCSI, Ireland
Development of an induced pluripotent stem cell spinal cord scaffold system to investigate and promote spinal cord repair

12:15 – 13:00 LUNCH BREAK

SESSION 4: VIRTUAL HANDS ON SESSION

Discussion chairs: Jorge Oliver-De La Cruz (FNUSA-ICRC), **Soraia Fernandes** (FNUSA-ICRC), **Fabiana Martino** (FNUSA-ICRC)

13:00 –14:00 Virtual System Demonstration: ZEISS Lattice Lightsheet 7- Long-term Volumetric Imaging of Living Cells (detailed information found in page 9)
Moderation – Katerina Zertova
System Demonstration – Dr. Steffen Burgold

14:00 –15:00 TESCAN Amber virtual demonstration: a practical session on cellular ultrastructure investigation by advanced electron microscopy techniques (detailed information found in page 10)
System Demonstration - Dr. Jakub Javurek, Head of Applications

15:00 – 16:00 Rapid-fire poster presentations (15 min each video + 10 min Q&A)
15.00-15.20 Poster session: Disease Modelling (II)
15.30 – 15.45 Poster session: Disease Modelling (III)
15.55 – 16.10 Poster session: Cellular mechanosensing (I)
16.20 – 16.35 Poster session: Cellular mechanosensing (II)

Additional information about the posters presented in each session can be found in pages 7-8.

16:45 Final remarks

POSTER SESSIONS

THURSDAY JUNE 10, 2021

16.45-17.00 Poster session: Cell, tissue dynamics and patterning

Jaana Schneider, LBI, Austria - Cre mRNA is not transferred by EVs from endothelial and adipose-derived stromal/stem cells during vascular network formation

Martin Pesl, FNUSA-ICRC, Czech Republic - Arrhythmic events in clustered human pluripotent stem cell-derived cardiomyocytes

Nuno Neto, TCD, Ireland - Intracellular label-free detection of mesenchymal stem cell metabolism within a perivascular niche-on-a-chip

Shimaa Abdelaleem, NIMS, Japan - Impact of extracellular matrix ligand densities on TGF-β-induced EMT

Stefano Rizzi, CCM, Italy - Production of living pericardium material for personalized cardiac valve repair

17-10-17.25 Poster session: Materials for regeneration

Carolina Oliver-Urrutia, CEITEC-BUT, Czech Republic - Isolation of human oral mucosa stem cells for tissue engineering applications in combination with polyvinylpyrrolidone hydrogel

Paraskevi Kavatzikidou, FORTH, Greece - Anisotropic topography on biodegradable polymeric replicas mediate mouse stem cell focal adhesion, mechanotransduction and osteogenic differentiation

Josef Jaros, ICRC-FNUSA, Czech Republic - Creating lung tissue models utilizing 3D bioprinting

Matteo Solazzo, TCD, Ireland - Structural crystallisation of crosslinked 3D PEDOT: PSS anisotropic porous biomaterials to generate highly conductive platforms for tissue engineering applications

Phanee Manganas, IESL-FORTH, Greece - Understanding the mechanism of focal adhesion formation and intracellular signalling pathway activation on micropatterned polymeric replicas

17.35-17.50 Poster session: Disease Modelling (I)

Cristina Manferdini, IRCCS, Italy - Low-Intensity pulsed ultrasound stimulation enhances chondrogenic differentiation of ASCs in a 3D hydrogel

Sinead O'Rourke, TCD, Ireland - Inflammation in atherosclerosis: Investigating cholesterol crystals as potent drivers of M1 polarisation and metabolic reprogramming in primary human macrophages

Annalena Dittmann, University of Oulu, Finland - The Burden of Post-Translational Modification (PTM)-Disrupting Mutations in the Tumor Matrisome

Gabriele Addario, MERLN, The Netherlands - Towards a renal tubulointerstitium in vitro model

Soraia Fernandes, FNUSA-ICRC, Czech Republic - Extracellular matrix tumorigenic alterations in prostate cancer organoids

FRIDAY JUNE 11, 2021

15.00-15.30 Poster session: Disease Modelling (II)

Vladimir Vinarsky, FNUSA-ICRC, Czech Republic - YAP1 regulates cardiomyocyte contractility through regulation through LTCC channels

Federico Tidu, FNUSA-ICRC, Czech Republic - Calcineurin-NFAT signalling in human mesenchymal stromal cells drives ECM remodeling and anti-fungal response

Mattia Spedicati, Politecnico di Torino, Italy - 3D in vitro model of early-stage human cardiac fibrosis based on bioartificial scaffold

Gerardina Ruocco, Politecnico di Torino, Italia - *In vitro* engineered model of fibrotic cardiac tissue based on electrospun bioartificial scaffolds

Helen Kearney, Maastricht University, Netherlands - The effect of iPSC culture conditions prior to downstream kidney organoid differentiation

Ece Ergir, FNUSA-ICRC, Czech Republic - Generation and Characterization of Organotypic Cardiac Microtissues for Translational Research

15.30-15.50 Poster session: Disease Modelling (III)

Ana Milena Bermeo Noguera, Universidad Nacional de Colombia - Preclinical evaluation of autologous artificial connective tissue made with oral mucosa and skin fibroblasts to increase keratinized gingiva

Michele Fenu, Erasmus MC, The Netherlands - Environmental mechanical parameters influence deposition of ECM

Marek Černik, IBP, Czech Republic - Production of a microfluidic chip to study the CD44-Hippo pathway cross-talk

Meenakshi Suku, TCD, Ireland - Tuning macrophage polarization to model myocardial infarction in the generation of functional cardiac organoids

Jan Víteček, IBP, Czech Republic- Mechanobiological aspects of thrombolysis: in vitro study in vascular models

15.55 – 16.10 Poster session: Cellular mechanosensing (I)

Šimon Klimovič, CEITEC, Czech Republic - Covalently cross-linked hyaluronic acid BASED hydrogels with tunable properties for cell culturing

Stefania Pagliari, FNUSA-ICRC, Czech Republic - PSC mesoderm specification is controlled by YAP-TEAD1-driven cytoskeleton dynamics and intracellular tension

Ferran Lozano Juan, BiomimX Srl. / Politecnico di Milano, Italy - A novel heart-on-chip coupled with uniaxial mechanical stimulaton and an integrated electrical reading system for drug cardiotoxicity studies

Marco Cassani, FNUSA-ICRC, Czech Republic - Toward the understanding of bio-nano interactions through mechanobiology

Jaroslaw Jacak, University of Applied Sciences Upper Austria, School of Applied Health and Social Sciences, Linz- Austria- In-vitro model of the human blood vessel wall

16.20 – 16.35 Poster session: Cellular mechanosensing (II)

Tosca Roncada, University of Portsmouth, United Kingdom - Development of hybrid hydrogels for osteochondral regeneration

Jan Přibyl, CEITEC MU, Czech Republic - Cellular mechanosensing by means of atomic force microscopy

Mazaya Najmina, University of Tsukuba, Japan - Material Fluidity Promotes the Senescence of Breast Cancer Cells in a Fluidity-dependent Manner

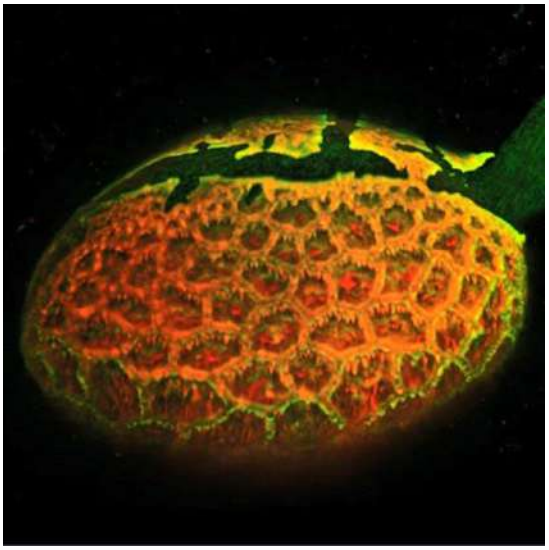
Lukas Kubala, FNUSA-ICRC, Czech Republic - Myeloperoxidase mediated alteration of endothelial function is dependent on its cationic charge

Virtual System Demonstration: ZEISS Lattice Lightsheet 7

Long-term Volumetric Imaging of Living Cells

The complexity of life is the result of the dynamic interplay within and between molecules, organelles, cells and tissues. Deciphering the underlying processes and principles has always been the most prominent pacer, driving technological breakthroughs in microscopy. Our portfolio allows scientists to capture all these levels of complexity with maximum gentleness. High-speed volume acquisition with minimal light exposure and almost isotropic resolution is achieved by novel ZEISS Lattice Lightsheet 7.

In this live demonstration, we will summarize the technical background of lattice lightsheet imaging and applications in fluorescence microscopy. The presentation will be followed by a Q&A session.



Key Learnings

Gentle long-term imaging of living specimen

High-speed volumetric imaging

Nearly isotropic resolution for high quality 3D visualization & quantification

© Sample courtesy of R. Whan, UNSW, Sydney, Australia

Speakers

Moderation – Katerina Zertova
(Regional Marketing Manager for Europe, Middle East, and Latin America)

System Demonstration – Dr. Steffen Burgold
(Application Specialist, ZEISS Microscopy Customer Center Europe)

Steffen Burgold studied biotechnology at the University of Applied Sciences Jena, Germany and worked as a process engineer at the startup company Novosom AG in Halle, Germany. In his postgradual master studies in photonics he emphasized on light microscopy techniques and did his thesis at the Charité, Berlin with Prof. Michael Schaefer. He moved on with his PhD and postdoc in neurobiology (Alzheimer disease) with Prof. Herms at the University of Munich and the German Center for Neurodegenerative Diseases, Germany. Here, Steffen established a correlative workflow from intravital microscopy to FIB-SEM imaging of the brain ultrastructure. Since 2017 Steffen has been working as application specialist in the ZEISS Microscopy Customer Center Europe.

TESCAN Amber virtual demonstration: a practical session on cellular ultrastructure investigation by advanced electron microscopy techniques

TESCAN is a global supplier of scientific instruments. The company is building its reputation and brand name in the field of designing and manufacturing scanning electron microscopes and system solutions for different applications.

The company is focused on research, development and manufacturing of scientific instruments and laboratory equipment such as:

- scanning electron microscopes
- supplementary accessories for SEMs
- light optical microscopy accessories and image processing
- special vacuum chambers and custom systems
- detection systems
- scientific hardware and software development

Speaker:

Mgr. Jakub Javůrek, Ph.D.

Head of Applications - Life Science TESCAN ORSAY HOLDING a.s.

BOOK OF ABSTRACTS

Virtual TERMIS workshop: "Cell-Matrix Interaction and Mechanobiology in Regenerative Medicine"

June 10-11, 2021

ORAL PRESENTATION 1

Influence of topography of micro-patterned Si surfaces on PC12 neural cell differentiation: implication of mechanotransduction pathways

Papadimitriou Lina[1*], Karagiannaki Anna[1,2], Emmanuel Stratakis[1,2], Ranella Anthi[1]

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In the field of tissue engineering and regenerative medicine one of the main goals is to design materials than can promote the development and/or the regeneration of neuronal tissue. Today, novel micro- and nano-fabrication techniques can create local cellular microenvironment to closely mimic the physiological and pathological environment within tissues and/or organs. Although a growing body of literature support the hypothesis that the substrate topography influences the cell proliferation and differentiation, the underlying mechanisms are, at present, poorly understood. The current study aims to link topographical cues with intracellular signaling pathways in neuronal cells and more specifically with molecules of the mechanotransductionI machinery[2].

Femto-second laser have been used to create Si substrates decorated with micro/nano topographies. PC12 cells treated with NGF differentiate well on flat Si and on Si decorated with microcones with low and medium roughness but they do not diffrentiate on the high roughened pattern[1] (**fig. 1**). Using Western blot analysis, we showed no significant differencies in the activation of the intracellular pathways of ERK/MAPK and PI3/AKT therefore we focused on mechanotrunsduction pathways. By SEM, confocal and Two-photon microscopy, we have found that as the substrate's roughness increases the number of Focal adhessions (Vinculin staining) decreases especially at the growth cone areas, the activation of Myosin light chain (pMLC-II staining) decreases and YAP nuclear translocation also decreases. We believe that in the high roughened substrate cells fail to stabilize growth cone formation and this leads to low differentiation ratio.

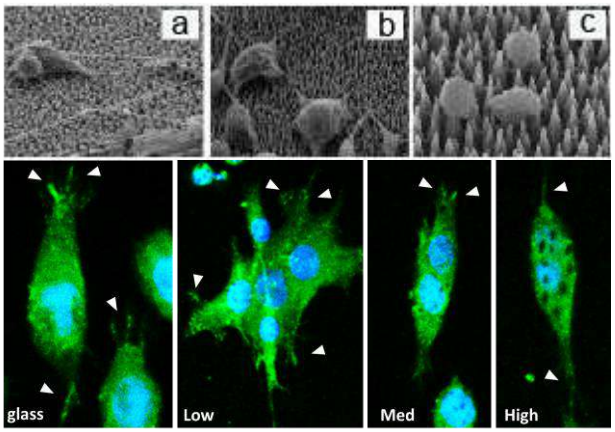

Figure 1: Upper panel: SEM images of PC12 cells treated with NGF on Si substrates decorated with microcones with different roughness (a: low roughness, b: medium roughness and c: high roughness). Lower panel: Focal adhesions (Vinculin) of PC12 cells treated with NGF for 24 hrs.

These findings are a significant contribution for the understanding of cell-surface interaction and especially the mechanisms through which cells "sense" and adapt to the surface and activate specific intracellular signals that control neural cell differentiation. The ability to control neuronal cell responses through the topography of the underlying substrates could be a useful tool for tissue engineering applications.

Acknowledgments

This work was supported by the European Horizon 2020 - FET OPEN Program under Grant No. 829060 ("NeuroStimSpinal"; URL: www.neurostimspinal.eu/")

References

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[2] Neuronal contact guidance and YAP signaling on ultra-small nanogratings, Ilaria Tonazzini, Cecilia Masciullo, Eleonora Savi, Agnese Sonato, Filippo Romanato & Marco Cecchini, Scientific Reports **2020** 10, 3742.

ORAL PRESENTATION 2

Convergence of Hippo/TGF-β pathways in mechanical activation of vein aorto-coronary bypass failure

G. Garoffolo[1*], G. Cassanmagnago[2], A. Thomas[3], M. S. Ruiter[1], M. Carrara[4], R. Vono[5], C. Saccu[1], M. Argrifoglio[6], M. Soncini[7], G. B. Fiore[7], F. Martelli[4], G. Condorelli[2], P. Madeddu[3], G. Spinetti[5], M. Pesce[1]

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Implantation of saphenous vein (SV) grafts in coronary position determines vessel wall remodeling, intimal hyperplasia and graft failure. This causes recurrence of cardiac ischemia in patients with coronary artery disease. We have recently found that experimentally exposing human SV to coronary-like counter-pulsed high flow/pressure regime induces release of matricellular protein Thrombospondin-1 (TSP-1) by resident smooth muscle cells due to cell strain dependent effects. This release recruits adventitial resident progenitors (SVPs) to a pro-fibrotic phenotype associated to stenosis of the coronary bypass in a large animal model of SV carotid interposition [1]. In the present work, we show the cooperation of TSP-1, a known component of the TGF-β *pro*-fibrotic pathways, with intracellular pathway activated by strain sensing in fibrotic differentiation of SVPs. Human SVs for tissue/cell culture were obtained during coronary artery bypass or SV stripping surgery. SVs were conditioned mechanically with a dedicated coronary pulse duplicator up to two weeks [2]. SVPs were derived as in [3], and subjected to uniaxial strain stimulation (10% deformation; 1Hz) using a commercial platform (FlexCell). Cell responses were analyzed with immunofluorescence, Western Blot, Q-RT-PCR, migration assays and RNA-seq profiling. SVPs mechanosensitivity was assessed by analysis of nuclear alignment and cell shape index/spread areas after straining. Mechanically stimulated cells showed a significant increase in their basal motility, as verified by migration assays in the presence of medium supplemented with 10% serum. A gene enrichment analysis of transcripts up/downmodulated by mechanical stress revealed an involvement of the HIPPO/YAP/TEAD and TGF-β/SMAD transcriptional circuitries in cells stimulated with the cyclic strain. Indeed, mechanical stimulation increased significantly the nuclear translocation of YAP and its transcriptional activity. This was associated to an increase in direct targets expression and it was mediated by formation of YAP/TEAD transcriptional complex, as verified by co-immunoprecipitation. In order to mechanistically correlate the effect of mechanical stress to YAP function, we inhibited the intracellular transmission of mechanical forces by treating cells with a drug interfering with actin polymerization (Forskolin, FRSK) and another molecule preventing association of YAP/TAZ complex with DNA binding protein TEADs (Verteporfin, VTP). Treatment with FRSK determined a completely reversible relocation of YAP from the nucleus to the cytoplasm, and this decreased expression of canonical target genes, as verified by Q-RT-PCR. Treatment with VTP caused a similar downregulation of YAP targets, in the presence of cytoskeleton tensioning. The two inhibitors also inhibited SVPs motility in migration assays. Convergence of the Hippo and TGF-β/TSP-1 pathways was confirmed by culturing SVPs in the presence of TGF-β and TSP-1, alone or in combination, followed by analysis of YAP target genes and fibrosis markers expression. Data showed that in the presence of TGF-β, with or without TSP-1, YAP-dependent targets were upregulated. Conversely, the two factors, only in combination, increased above controls the level of nuclear-localized YAP and that of pSMAD2/3, which are the canonical TGF-β signaling transducers. In addition, an upregulation of collagen 1A transcripts and consequent increased release of collagen content was observed in SVPs cultured in presence of TGF-β/TSP-1. VTP restored the quantity of soluble collagen to the basal levels, even in the presence of TGF-β/TSP-1. Finally, nuclear localization of YAP was detected in human SVs exposed to coronary flow mechanics *in vitro* as well as in arterialized SVs in a pig model of carotid interposition. Our data establish for the first time a cooperation between the matricellular changes in the SV wall exposed to coronary mechanics, and paracrine effects mediated by convergent activation of Hippo/TGF-β pathways in failure of the human aorto-coronary bypass.

Keywords: YAP, Mechanical strain, Aorto-coronary bypass,

References

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ORAL PRESENTATION 3

hnRNPC mediates the mechanoregulation of mRNA homeostasis in heart failure

Fabiana Martino[1,2,3], Ana Rubina Perestrelo[1], Vaclav Hejret[4,5], Nandan Mysore Varadarajan[4], Helena Durikova[1], Vladimir Horvath[1,6], Francesca Cavalieri[7,8], Frank Caruso[7], Waleed S. Albihlal[9], André P. Gerber[9], Mary A. O'Connell[4], Stepanka Vanacova[4], Stefania Pagliari[1,3], Giancarlo Forte[1,3*].

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Alterations in the composition and mechanics of extracellular matrix (ECM) contribute to the progression of cardiac diseases, impairing cardiac cell function, thus leading progressively to organ failure. To cope with the mechanical turmoil, cardiomyocytes adopt a hypertrophic compensatory mechanism, consisting in the re-expression of fetal contractile proteins via transcriptional and post-transcriptional processes, such as alterative splicing (AS). As a result, RNA binding is among the main molecular functions affected in diseased hearts. Whether mRNA homeostasis can be directly affected by ECM remodeling remains so far poorly investigated.

Here, we show that heterogeneous nuclear ribonucleoprotein C (hnRNPC), a core spliceosome associated protein, is upregulated in the pathological heart, independently from the etiology of the disease. We demonstrate that hnRNPC localizes at the contractile apparatus of cardiomyocytes in the failing human heart where, upon ECM pathological remodeling, it participates to the localized translation of sarcomeric transcripts. Moreover, we observe that hnRNPC localization is affected by the ability of cells to develop intracellular tension and spread within the surrounding environment.

Interestingly, mechanically-induced hnRNPC displacement or alteration in its expression affect the alternative splicing of mechanosensitive genes, including the Hippo pathway effector YAP1. Given the relevance of this mechanically activated protein in cardiac pathologies, we suggest that pathological ECM remodeling serves as a switch in RNA metabolism by tuning distribution and function of the splicing regulator hnRNPC. These findings offer new insights on the impact of mechanics on RNA homeostasis in pathological conditions.

ORAL PRESENTATION 4

The Matricellular Protein SPARC Modulates Load-sensing in Tendons

T. Wang[1,2†], A. Wagner[3,4†], R. Gehwolf[3,4†], W. Yan[5†], F.S. Passini[6,7], S. Senck[8], J.G. Snedeker[6,7], H. Tempfer[3,4], Q. Jiang[5], M.H. Zheng[1], A. Traweger[3,4*]

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[9]Computed Tomography Research Group, University of Applied Sciences Upper Austria, Wels, Austria.
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Tendons and tendon interfaces have a very limited regenerative capacity, rendering their injuries clinically challenging to resolve. Tendons sense muscle-mediated load, however our knowledge on how loading affects tendon structure and functional adaption remains fragmentary. We have previously shown that Secreted Protein Acidic and Rich in Cysteine (SPARC) expression is reduced in tendons of old mice, resulting in impaired cell-ECM binding of tendon-resident stem and progenitor cells (TDSCs) and increased lipid accretion in tendon tissue, a hallmark of tendon degeneration [1]. We now provide evidence that the matricellular protein SPARC is critically involved in the mechanobiology of tendons and is required for tissue maturation, homeostasis, and enthesis development [2]. We show that tendon loading at the early postnatal stage leads to tissue hypotrophy and impaired maturation of Achilles tendon enthesis in Sparc$^{-/-}$ mice. Treadmill training revealed a higher prevalence of spontaneous tendon ruptures and a net catabolic adaptation in Sparc$^{-/-}$ mice. Tendon hypoplasia was attenuated in Sparc$^{-/-}$ mice in response to muscle unloading with botulinum toxin A. In vitro culture of Sparc$^{-/-}$ three-dimensional (3D) tendon constructs showed load-dependent impairment of ribosomal S6 kinase (S6K) activation, resulting in reduced type I collagen synthesis. Further, functional calcium imaging revealed that lower stresses were required to trigger mechanically induced responses in Sparc$^{-/-}$ tendon fascicles. To underscore the clinical relevance of the findings, we further demonstrate that a missense mutation (p.Cys130Gln) in the follistatin-like (FS) domain of SPARC, which causes impaired protein secretion and type I collagen fibrillogenesis, is associated with tendon and ligament injuries in patients. Taken together, our results demonstrate that SPARC is a key extracellular matrix protein essential for load induced tendon tissue maturation and homeostasis.

Keywords: SPARC, tendon, enthesis, tissue homeostasis, rotator cuff injury, AKT, S6K, mechanosensing

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Study of the combined effect of shear stress and laser-patterned topography on Schwann cells' behavior

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Due to the dysfunctional spontaneous recovery of the nervous system after injury, the successful guidance of neuronal outgrowth, *in vitro*, is mandatory for neurogenesis. Therefore, the development of successful methods to guide neuronal outgrowth in a controllable manner, *in vitro*, is of high significance [1]. The present work aims to present a first study of the combined effect of shear stress and laser-patterned topography on Schwann (SW10) cells' behavior under dynamic culture conditions attained via continuous flow.

For this purpose, a precise flow controlled microfluidic system with specific custom-designed chambers incorporating laser-microstructured polyethylene terephthalate (PET) culture substrates comprising microgrooves [2] was developed [3]. The microgrooves were positioned either parallel or perpendicular to the direction of the flow inside the chambers and the response of SW10 cells was evaluated in terms of growth, orientation, and elongation. Additionally, the cell culture results were complemented with computational flow simulations aimed to precisely calculate the shear stress values. Our results demonstrated that wall shear stress gradients may be acting either synergistic or antagonistic to substrate groove orientation in promoting guided morphologic cell response when microgrooves are placed parallel or perpendicular to the mean flow direction respectively [3].

The ability to guide the outgrowth of SW10 cells, *in vitro,* via flow-induced shear stress and surface topography, could be potentially useful in the fields of neural tissue engineering with the ultimate goal of the creation of autologous graft substitutes for nerve tissue regeneration.

Acknowledgments

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ORAL PRESENTATION 6

Nucleoskeletal organization is influenced by YAP-dependent extracellular matrix remodelling

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Detecting mechanical cues, including the physicochemical properties of the extracellular matrix (ECM) is an essential aspect of cellular biology. Therefore, cells possess several systems to sense and adjust their response accordingly. Lamin A is a mechanoresponsive nucleoskeletal component, which provides mechanical support to the nucleus and modulates its stiffness [1]. Another well-studied mechanoregulated protein is YAP, whose nuclear presence correlates with cell contractibility. While recent reports [2,3] indicate that Lamin A contribution to nuclear integrity is required for a correct YAP regulation, an inverse effect (i.e the effect of YAP activity in lamin A and nucleoskeletal organization) has not yet been established.

Using the CAL51 breast cancer line as a model, we demonstrated that YAP depletion led to altered nuclear structure and composition, with a reduction and hyperphosphorylation of Lamin A protein. These changes were accompanied by a reduction of cellular stiffness. Interestingly, YAP-KO cells also lose the ability to remodel, degrade and consequently generate their own ECM, while, in contrast, YAP overexpression induced higher ECM deposition. When YAP KO cells were seeded onto the ECM produced by WT or YAP overexpressing cells, they only significantly recovered nucleoskeletal morphology and lamin/phospho-lamin nuclear levels in the ECM produced by cells with a higher YAP levels. This rescue was mediated by intermediate filaments and was independent of actomyosin and the microtubular cytoskeleton.

Our results depict a new mechanoparacrine effect, by which YAP activity, in modifying ECM deposition, can alter the cellular and nucleoskeletal mechanics of surrounding cells, independently of their own YAP intracellular regulation.

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Development of an induced pluripotent stem cell spinal cord scaffold system to investigate and promote spinal cord repair

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Following spinal cord injury, trophic astrocytes become 'reactive'[1] and injurious to neurons as well as contributing to scar tissue formation[2], preventing axon regrowth through the lesion site to restore sensorimotor function. Developing strategies that mitigate astroglial responses while also promoting neuronal survival and axonal regrowth may be critical for successful repair in the lesion site. Building on expertise in developing peripheral nerve guidance scaffolds[3] we aimed to create a dual function biomimetic tissue scaffold for i) 3D spinal cord modelling of neuronal and glial interaction and ii) a trophic implant capable of bridging the lesion cavity. Screening of native central nervous system extracellular matrix proteins revealed that collagen-IV and fibronectin combined, enhanced motor neuron (39%, $p<0.01$) and spinal cord astrocyte outgrowth (70%, $p<0.05$). Collagen-IV and fibronectin also increased astrocyte process number ($p<0.05$), metabolic activity ($p<0.05$) and decreased levels of reactivity ($p<0.05$). Subsequently, 3D hyaluronic acid scaffolds with aligned microarchitecture and mechanical properties matching the spinal cord, functionalized with collagen-IV and fibronectin were fabricated. Spinal cord astrocytes cultured in scaffolds ranging from soft (0.9kPa) to stiff (3kPa & 6.1kPa), with or without collagen-IV and fibronectin revealed that soft, collagen-IV/fibronectin functionalized scaffolds promoted phenotypes typical of resting cells, increased their metabolic activity and secretion of IL-10. Furthermore, soft scaffolds seeded with induced pluripotent stem cell (iPSC)-derived astrocytes and neurons promoted cell viability ($p<0.05$), outgrowth/infiltration ($p<0.0001$) and differentiation compared to stiffer scaffolds. Additionally, soft scaffolds encouraged the growth of iPSC derived spheroids 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 that a novel scaffold system with physiochemical and mechanical properties matching that of the spinal cord supports robust astrocytic and neuronal growth. These findings have implications for 3D modelling of astrocyte-neuronal interactions in an anatomically and physiologically relevant environment and for further development of scaffold therapeutics for promoting cord repair.

Keywords: Spinal Cord Injury, Stiffness, Matrix, Tissue Scaffold, Induced Pluripotent Stem Cells

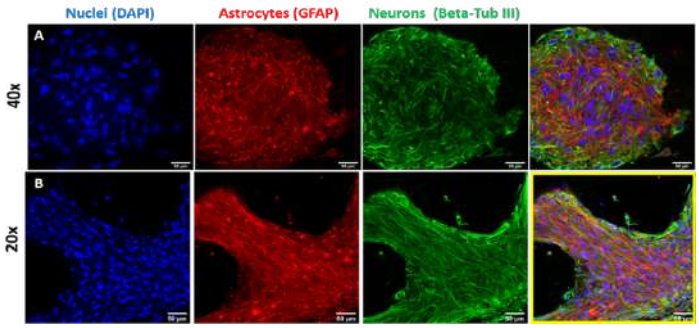

Figure 1. Softer scaffolds support induced pluripotent stem cell derived neurons and astrocytes. *Lineage restricted iPSC derived astrocyte precursor cells form multicellular structures (A) that can, in turn, form dense multicellular networks that display spatial differences in cell type throughout the scaffold following 7 days in culture (B). Scale bars = 50µm.*

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Cre mRNA is not transferred by EVs from endothelial and adipose-derived stromal/stem cells during vascular network formation

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Co-culture systems employing adipose tissue-derived mesenchymal stromal/stem cells (ASC) and endothelial cells (EC) represent a widely used technique to model vascularization [1-4]. Within this system, cell-cell communication is crucial for the achievement of functional vascular network formation. Extracellular vesicles (EVs) have recently emerged as key players in cell communication by transferring bioactive molecules between cells [5-7]. In this study we aimed to ad-dress the role of EVs in ASC/EC co-cultures by discriminating between cells which have received functional EV cargo from cells which have not. Therefore, we employed the Cre-loxP system, which is based on donor cells expressing the Cre recombinase, whose mRNA was previously shown to be packaged into EVs and reporter cells containing a construct of floxed dsRed upstream of the eGFP coding sequence [8]. The evaluation of Cre induced color switch in the reporter system via EVs indicated that there is no EV-mediated RNA transmission either between EC themselves or EC and ASC. However, since Cre mRNA was not found present in EVs, it remains unclear if Cre mRNA is generally not packaged into EVs or if EVs are not taken up by the utilized cell types. Our data indicate that this technique may not be applicable to evaluate EV-mediated cell-to-cell com-munication in an in vitro setting using EC and ASC. Further investigations will require a functional system showing efficient and specific loading of Cre mRNA or protein into EVs.

Keywords: extracellular vesicles; endothelial cells; cell-cell communication; Cre-loxP system; co-culture

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Arrhythmic events in clustered human pluripotent stem cell-derived cardiomyocytes

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Aminophylline is a methylxanthine bronchodilator with a documented proarrhythmic action. We aimed to describe changes in rhythm pattern of spontaneously beating human pluripotent stem cell-derived cardiomyocytes (hPSC-CM). Moreover the inotropic changes were never before studied in hPSC-CM.

Methods: hPSC-CM were differentiated in clusters[1-3] basic biomechanical parameters, such as the average value of contraction force and the beat rate (BR), were assessed by atomic force microscopy (AFM) as previously described[1,2]. Cells were stabilized in Tyrode solution (baseline) and applied were increasing concentrations of aminophylline (10 µM, 100 µM, 1 mM, and 10 mM).
Results: the 10mM aminophylline significantly increased beat rate (BR) in comparison with the lower concentrations. There were no significant differences in inotropic effects of aminophylline on the hPSC-CMs between all groups and concentrations. Number of measured clusters underwent atypical arrhythmic pattern - termed "stop&go effect" - presenting as a series of fast BR episodes (the equivalent of tachycardia) followed by inactivity. This effect occurred during aminophylline treatment with various concentrations.

Conclusions: an aberrant cardiomyocyte response to aminophylline was observed, suggesting an arrhythmogenic potential of the drug. Our data represent a missing link between the arrhythmic events related to the aminophylline/theophylline treatment in clinical practice and subcellular mechanisms of methylxanthine arrhythmogenesis. AFM combined with hPSC-CM serve as a robust platform for direct drug effects screening.

Keywords: iPSC; hESC; cardiomyocytes; pulmonary drug screening; drug cardiotoxicity; atomic force microscopy; biomechanical properties; arrhythmogenic effects; methylxanthines aminophylline

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Intracellular label-free detection of mesenchymal stem cell metabolism within a perivascular niche-on-a-chip

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Adult progenitor cell populations are present in a microenvironment named perivascular stem cell niche that presents specific conditions to maintain their self-renewal and multi-lineage potential [2]. Crisan, Corselli, Chen and Peault. Here, physicochemcical factors such as oxygen concentration, nutrient availability, signaling factors as well as pH, shear stress or temperature contribute to its homeostasis [3]. Discher, Mooney and Zandstra. Mesenchymal stem cells (MSCs), which are emerging as key players in the modulation of the overall niche response and organization are among the different cell population involved in the niche homeostasis [4]. Several reports have examined the effect of flow and sheer stress on cell differentiation an mineralisation but reports focusing on the metabolis states of cells in such conditions are lacking [1]. Predictive modelling was employed to engineer a declining gradient in oxygen tension and validated using a previously calibrated non-invasive imaging technology applicable to this miniaturised optically accessible bioreactor (MOAB). Mass transport analysis was implemented to predic oxygen tension profile with consideration of h-MSCs oxygen consumption. The cellular consumption rate was modelled as an oxygen sink by defining a steady state flux, dependent on cell density, along the cell adhesion surface of the MOAB chamber while assuming a uniform distribution of cells at a fixed reference cell density of 6 × 104 cells per cm2, the oxygen tension exponentially decreased with the flowrate due to the h-MSCs oxygen uptake. To assess and validate the predicted cell oxygen uptake, we performed fluorescence intensity based measurements inside the MOAB using an intracellular phosphorescent probe. A statistically significant increase in the ratio 630/460 from the inlet area of 1.09 ± 0.04, to 1.44 ± 0.10 in the middle, and finally 1.68 ± 0.17 in the outlet area was detected in the case of 0.5 µL min−1 while no detectable significant overall increase resulted in the case of 5 µL min−1. FLIM analysis of h-MSCs at these locations revealed a decreasing gradient in the average fluorescence lifetime while the protein bound NAD(P)H lifetime remains stable. The calculated average fluorescence lifetime for the inlet, middle and outlet are 1.545 ± 0.012 ns, 1.415 ± 0.070 ns, 1.325 ± 0.045 ns, respectively. In addition, the middle and outlet values have statistically significantly decreased when compared to inlet values. The computer modeling and FLIM measurements were then validated using mitochondria and glucose uptake stainings (TMRM and 2-NBDG, respectively). These confirmed the metabolic shifts of h-MSCs at different areas, as a higher decrease of glucose uptake and mitochondria size was observed using lower flow rates. This toolbox is an innovative in vitro platform that can allow tunable and measurable oxygen tension gradients that can achieve appreciable impacts on cellular metabolism. This advanced transient microenvironment can recapitulate aspects of intravital niches providing a reliable tool for diseases modelling and drugs screening.

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Impact of extracellular matrix ligand densities on TGF-β-induced EMT

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Epithelial-mesenchymal transition (EMT) is a fundamental physiological process that occurs naturally during embryogenesis, tissue repair, or during ailing condition as in cancer metastasis. It is well-known how this transition is governed by pure soluble factors. However, it becomes identified rather recently, the cellular responses to the soluble factors can be altered by the properties of underlying ECM. For instance, following TGF-β stimulation, MDCK cells grown on stiff substrates underwent EMT whereas cells grown on compliant substrates underwent apoptosis (1). On the other hand, there are limited reports on the contribution of the quantitative difference in the ECM composition to soluble factor-induced EMT. Therefore, we investigated the regulation of the EMT induction by TGF-β in relation to their underlying ECM concentration, the understanding of this crosstalk is essential especially when the variations in ECM concentration have proved to solely control the acquisition of epithelial/mesenchymal characteristics as we previously reported(2,3). For this purpose, well-controlled cRGD-functionalized surface were prepared by mixing of two disulfides bearing cell adhesive (cRGD) peptide and cell-repellent EG6 in different mixing ratios (1:100, 1:10k). The presence of EG6 group prevents native and nonspecific protein adsorption to the substrate thus minimizing cellular ECM remodeling. MDCK cells were cultured on these substrates with low and high ligand density for 5 hrs, followed by TGF-β stimulation for 18 hrs. After that, the acquisition of mesenchymal phenotype was morphologically and biochemically examined and compared to cells cultured in tissue culture plates (TCP). For instance, the appearance of actin stress fibers for cells cultured in dense cRGD was observed upon the stimulation by TGf-β, however, to a lesser degree compared to the cells cultured in sparsely immobilized cRGD (Figure-1). Furthermore, the increase in mesenchymal proteins as N-cadherin, vimentin and snail were found higher after TGF-β treatment for cells grown on low cRGD substrates compared to cells grown on high cRGD substrates and even compared to cells cultured on TCP. Finally, we identified that a certain sparse concentration of cRGD would enhance the cellular responses to TGF-β compared to others or even TCP. These results may provide new insights to profoundly understand the correlation between the components of tumor microenvironment in a more complex nature.

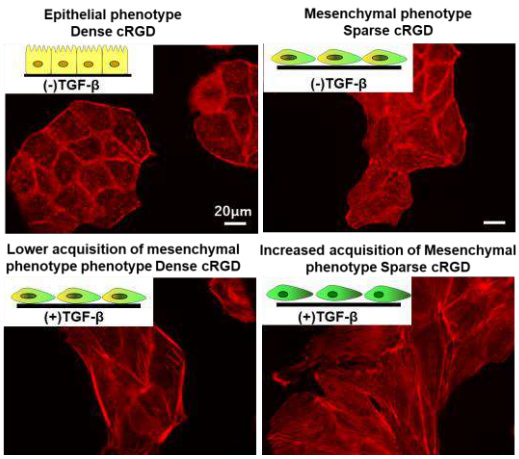

Figure-1: Actin stained with phalloidin (red fluorescence) upon culture of MDCK on densley and sparsely immobilized cRGD substrates (-/+) TGF-β.

Keywords: EMT, TGF-β, ECM chemical cues, ligand concentration.

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Production of living pericardium material for personalized cardiac valve repair

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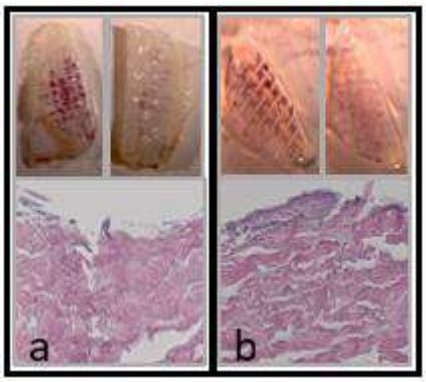
The number of heart valve procedures is expected to triple by 2050 [1] with an increase in the impact on public health. Mechanical or biological artificial prostheses are not ideal as valve replacement devices. In fact, they are prone to thromboembolic complications (mechanical) or structural deterioration (biological), with recurrence of valve failure in the mid/long term. In the present contribution, based on a method already tested at a laboratory scale [2, 3], we describe the first results of an experimental program to obtain a fully cellularized tissue using animal (bovine)-derived decellularized pericardium and human adipose tissue-derived mesenchymal cells for a personalized therapy of the aortic valve.

Decellularization procedure, consisting of mechanical removal of fat tissues, cell lysis by osmotic shock and subsequent removal of debris with both SDS and Triton and of nucleic acid material with DNase, was previously validated on porcine pericardium for increase of tissue permeability [2, 3]. Given that bovine pericardium has a substantially higher thickness and possibly different arrangement of extracellular matrix, perfusion tests were performed on the tissue before and after decellularization. Results showed a significantly increased permeability in decellularized *vs.* native pericardium (4.87721E-14±1.207e-014 *vs.* 2.222e-013±5.223e-014, mean±SE *vs.* mean±SE; P=0.0234; n=3). This confirmed the possibility to employ a direct perfusion bioreactor to reintroduce cells into the decellularized material. Circular patches (1cm in diameter) of decellularized material were cut with surgical puncher and set into a conventional perfusion system (U-Cup), for preliminary recellularization experiments using cells Human Mesenchymal Stem Cells (hMSC, Lonza) (quantity: $0.65*10^6$ cells/cm^2; seeding phase: 5ml/min, culture phase: 0.03 ml/min; time: D3, D7).

These data confirm provisionally the feasibility of bovine pericardium recellularization using human adipose-derived mesenchymal cells. Considering that these cells can be easily expandable from biopsies of subcutaneous fat from virtually every donors and that our decellularization procedure removes Xenoantigens (i.e. αGAL) known to cause immune rejection, we propose this method as the first personalized approach in valve tissue engineering, amenable for adult/pediatric valve reconstruction, using scaled up perfusion systems and GMP compliant procedures.



Results at D3 (a) and D7 (b) showed good seeding efficiency and cell growing in time.

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Isolation of human oral mucosa stem cells for tissue engineering applications in combination with polyvinylpyrrolidone hydrogel

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Oral tissues have become a valuable source of mesenchymal stem cells (MSCs) due to the easy accessibility that allows a less invasive procedure. Moreover, the epithelium of oral mucosa has an important regenerative potential that reduces the recovery time after biopsy harvesting. Other characteristics of the MSCs of oral origin are that they show immunosuppressive properties and an anti-inflammatory function that may aid in tissue repair. Moreover, similar to other MSCs, oral MSCs can differentiate into osteoblasts, adipocytes, and chondrocytes under specific in vitro conditions [1,2]. In this work, the isolation of human oral mucosa stem cells (hOMSCs) is explored. Human donors were previously informed about the procedure, provided consent for biopsy and cell harvest. Samples were obtained during oral surgeries for clinical reasons, from the retromolar region and maxillary tuberosity of oral mucosa. The isolated hOMSCs expressed typical markers of mesenchymal stem cells in more than 95 % of the population and had fusiform morphology [3].

In addition, polyvinylpyrrolidone (PVP) scaffolds were produced by a heat-activated polymerization and lyophilization to allow the three-dimensional culture, transport, and delivery of hOMSCs. To understand the swelling behaviour of PVP hydrogels, swelling kinetics was studied in phosphate-buffered saline (PBS) and Dulbecco's modified eagle's medium (DMEM) at 37 °C. The swelling of the hydrogel was higher in the DMEM (14.50 ± 1.87 %) than in PBS (8.25 ± 0.59 %). Furthermore, the swollen PVP hydrogel showed a storage modulus similar to oral mucosa and elastic solid rheological behaviour without sol transition. Therefore, it is mechanically stable under cell culture conditions. According to the porosity evaluation, PVP scaffolds showed a heterogeneous distribution of oval-like pores with broad size distribution (5 to 180 µm, d50 = 65 µm), which may be suitable for skin regeneration and reconstruction of teeth supporting structures. An in vitro hemocompatibility assay was performed in contact with human blood according to the ISO standard 10993-4. The results showed that PVP is not toxic for human red blood cells. Furthermore, direct contact cytotoxicity assay performed according to ISO standard 10993-5 showed that PVP has no negative effect on cell metabolic activity of hOMSCs after 10 days of culture [3, 4].

Keywords: polyvinylpyrrolidone, hydrogel, oral stem cell, tissue engineering.

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Anisotropic topography on biodegradable polymeric replicas mediate mouse stem cell focal adhesion, mechanotransduction and osteogenic differentiation

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Engineered microenvironments are offering mechanistic insights into how the extracellular matrix (ECM) and physical forces regulate stem cells, revealing how these control self-renewal, adhesion, proliferation and differentiation potentials. The cells sense ECM mechanics (mechanotransduction).

Ultrafast pulsed laser irradiation is considered as a simple microfabrication method to produce structures controlling the structure geometry and pattern regularity[1]. Such structures with an anisotropy discontinuous topographical nature could enhance cellular growth and alignment (eg neuronal[2,3]). Soft lithography has been successfully used to transfer well-defined micro-sized patterns from silicon to polymeric surfaces allowing the in-depth study on cell behavior[4].

Here, the ultrashort laser irradiation technique fabricated a series of silicon (Si) structures with different anisotropic discontinuous patterns at micro-nano scale. Soft lithography was used to successfully reproduce positive replicas of biodegradable polymers (PLGA with different molecular weight and PCL) from the Si structures. The morphological, wetting and degradation properties of the polymeric replicas were determined by Scanning Electron Microscopy (SEM), contact angle, and weight loss measurements. Mouse Mesenchymal Stem Cells C57BL/6 was the cell line to be investigated. Cell mechanotransduction was analyzed via the focal adhesion activity, cytoskeleton organization (shape) and cell nuclear profile on the replicas. The anisotropic topographies affected MSC cell fate and differentiation. The surface roughness mediated MSCs mechanotransduction and differentiation. Cellular morphology and nuclear mechanics was influenced by the chemical composition and degradation rate. The ability of our technique to control the cellular behavior and create cell patterns could be potentially useful in understanding disease pathogenesis and for the development of patient-specific applications.

Keywords: anisotropic topography, PLGA, PCL, MSCs, cell mechanotransduction, osteogenic differentiation

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Creating lung tissue models utilizing 3D bioprinting

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INTRODUCTION: 3D cell culture techniques provide much deeper insights to cellular behavior and in comparison to standard planar cultivation 3D environment promotes cell organization into complex tissue- and/or organ-like structures. 3D bioprinting gives us the possibility to create tissue structures of simple one-layered organization up to complex 3-dimensional models. Lung tissue is typical by its highly branched morphology and spectrum of multiciliated, secretory cells, basal cells and alveolar cells. There had been progress in differentiation of human pluripotent stem cells and production of organoids, but these strategies are not capable of producing full spectrum of lung cells with their typical distribution and organization. We focus on preparation of lung tissue models utilizing lung progenitors and supportive cells printed within various hydrogels. One of them is alginate, which is broadly applied material and human stem cells printed in alginate hydrogels can be grown for a prolonged period of time, however alginate lacks bioactive motifs which decelerate cell expansion rate and migration.

METHODS: We were therefore fine-tuning properties of printed hydrogels – alginate modified by extracellular matrix proteins, synthetic peptides and growth factors as well as mechanical properties to stimulate specific response of lung organoids (proliferation, differentiation, migration, fusion). We designed and printed branched mono- and multi-layered 3D structures utilizing 3D bioprinter Bioscaffolder3 (Gesim).

RESULTS: We present creation of lung tissue models by utilizing 3D bioprinting in basic and complex structures. We optimized conditions for fusing lung organoids and building tubular structures with spectrum of differentiated lung specific cells causing minimal stress and control their behavior during in vitro culture.

Keywords: Biomaterials, Tissue models, 3D bioprinting, Cell organization, Organoids

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Structural crystallisation of crosslinked 3D PEDOT:PSS anisotropic porous biomaterials to generate highly conductive platforms for tissue engineering applications

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Conductive polymers (CPs) are enabling the achievement of smarter electrode coatings, piezoresistive components within biosensors and scaffolds for tissue engineering. Despite their advances in recent years, there exist still some challenges, such as long-term stability in physiological conditions, adequate long-term conductivity and optimal biocompatibility. Additionally, another hurdle to the use of these materials is their adaptation towards three-dimensional (3D) scaffolds; a feature that is usually achieved applying CPs as coating on a bulk material.

Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) is by far one of the most promising CPs in terms of its stability and conductivity, with the latter capable of being enhanced via a crystallisation treatment using sulphuric acid. In this work, we present a new generation of 3D electroconductive porous biomaterial scaffolds based on PEDOT:PSS crosslinked via glycidoxypropyltrimethoxysilane and subjected to a sulphuric acid crystallisation. The resultant isotropic and anisotropic porous scaffolds exhibited, on average, a 1000-fold increase in conductivity when compared with untreated scaffolds. Moreover, we also document precise control over pore microarchitecture, size and anisotropy with high repeatability to achieve mechanical and electrical anisotropy, while exhibiting adequate biocompatibility. These findings herald a new approach towards generating anisotropic porous biomaterial scaffolds with superior conductivity achieved through a safe and scalable post-treatment.

Keywords: electroconductive biomaterials, in vitro model, pedot:pss, microarchitecture control.

Understanding the mechanism of focal adhesion formation and intracellular signalling pathway activation on micropatterned polymeric replicas

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Cells respond to their surrounding environment through cues that they receive at the cell-substrate interface. These cues, usually provided by the extracellular matrix (ECM), can be mimicked through the use of nano- and micro-patterned surfaces. Ultrafast pulsed laser irradiation is routinely used to produce surfaces with controlled geometry and patterning. Laser-modified Si substrates have been shown to be effective in enhancing neuronal growth and alignment[1]. The laser-induced micro/nano topographies on Si surfaces can be successfully reproduced on various biocompatible and biodegradable polymers via soft-lithography techniques, enabling the comprehensive study of cellular behaviour for potentially implantable devices[2,3].

In this work, we have studied the way in which the topography and the material affect the cellular responses on a molecular level. To this end, mouse mesenchymal stem cells (MSCs) (C57BL/6 background) were cultured on various substrates and their response was investigated through the preparation of protein extracts, as well as total RNA extraction and qPCR analysis. We investigated the changes in the expression of genes and proteins involved in the formation of focal adhesion complexes (FACs). The intracellular pathways that control cellular adhesion and signal transduction were differentially activated in response to the cues received through the interactions of the cells with the surfaces on which they were grown. Our results add valuable information on the understanding of how mesenchymal stem cells interact with scaffolds, which has important implications for MSC-driven cell therapy applications[4].

Keywords: mesenchymal stem cells, focal adhesion complex, mechanosensing, topography, signalling

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Low-Intensity pulsed ultrasound stimulation enhances chondrogenic differentiation of ASCs in a 3D hydrogel

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Articular cartilage injuries have a limited potential to heal and, over time, may lead to osteoarthritis, an inflammatory and degenerative joint disease associated with activity-related pain, swelling, and impaired mobility [1]. Regeneration and restoration of the joint tissue functionality remain unmet challenges. Stem cell-based tissue engineering is a promising paradigm to treat cartilage degeneration [1]. In this context, hydrogels have emerged as promising biomaterials, due to their biocompatibility, ability to mimic the tissue extracellular matrix and excellent permeability [2]. Different stimulation strategies have been investigated to guarantee proper conditions for mesenchymal stem cell differentiation into chondrocytes, including growth factors, cell-cell interactions, and biomaterials [3]. An interesting tool to facilitate chondrogenesis is external ultrasound stimulation. In particular, low-intensity pulsed ultrasound (LIPUS) has been demonstrated to have a role in regulating the differentiation of adipose mesenchymal stromal cells (ASCs) [4]. However, chondrogenic differentiation of ASCs has been never associated to a precisely measured ultrasound dose. In this study, we aimed to investigate whether dose-controlled LIPUS is able to influence chondrogenic differentiation of ASCs embedded in a 3D hydrogel.

Human adipose mesenchymal stromal cells at $2*10^6$ cells/mL were embedded in a hydrogel ratio 1:2 (VitroGel RGD®) and exposed to LIPUS stimulation (frequency: 1 MHz, intensity: 250 mW/cm^2, duty cycle: 20%, pulse repetition frequency: 1 kHz, stimulation time: 5 min) in order to assess its influence on cell differentiation. Hydrogel-loaded ASCs were cultured and differentiated for 2, 7, 10 and 28 days. At each time point cell viability (Live&Dead), metabolic activity (Alamar Blue), cytotoxicity (LDH), gene expression (COL2, aggrecan, SOX9, and COL1), histology and immunohistochemistry (COL2, aggrecan, SOX9, and COL1) were evaluated respect to a non-stimulated control.

Histological analysis evidenced a uniform distribution of ASCs both at the periphery and at the center of the hydrogel. Live & Dead test evidenced that the encapsulated ASCs were viable, with no signs of cytotoxicity. We found that LIPUS induced chondrogenesis of ASCs embedded in the hydrogel, as demonstrated by increased expression of COL2, aggrecan and SOX9 genes and proteins, and decreased expression of COL1 respect to the non-stimulated control.

In conclusion, these results suggest that the LIPUS treatment could be a valuable tool in cartilage tissue engineering, to push the differentiation of ASCs encapsulated in a 3D hydrogel.

Keywords: Low-intensity pulsed ultrasound (LIPUS), Adipose mesenchymal stromal cells (ASCs), Hydrogels Chondrogenic differentiation.

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Inflammation in atherosclerosis: Investigating cholesterol crystals as potent drivers of M1 polarisation and metabolic reprogramming in primary human macrophages

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Atherosclerosis is a leading cause of heart failure, accounting for a third of deaths worldwide (1). Pro-inflammatory macrophages largely contribute towards advancement of this disease, causing disruption to the structural and mechanical integrity of atherosclerotic plaques (2). Current research aims to characterise key drivers of the M1 macrophage phenotype in atherosclerotic plaques. Cholesterol crystals, which are found to accumulate at both early and advanced stages of atherosclerosis; are known to drive inflammatory responses in macrophages (3, 4). However, the impact of cholesterol crystals on macrophage polarization has not yet been examined. Moreover, it has been demonstrated that macrophages in atherosclerotic plaques have a highly glycolytic profile which correlates with decreased plaque stability and increased incidence of rupture and thrombosis (5). There are, however, no studies to date linking cholesterol crystals to metabolic reprogramming. Therefore, the aim of this study is to examine the impact of cholesterol crystals in metabolic reprogramming and polarization of macrophages.

Primary human macrophages were treated with cholesterol crystals (500 µg/ml) over 24 hours in the presence/absence of the glycolytic inhibitor, 2-deoxyglucose (25 mM). mRNA expression was assessed by qPCR and cytokine production was assessed by ELISA. Macrophage metabolism was examined using fluorescence lifetime imaging microscopy (FLIM) and Agilent Seahorse assays. Mitochondrial morphology was assessed through confocal imaging.

The results of this study demonstrate that cholesterol crystals drive significant expression of M1 associated genes, while concomitantly downregulating expression of M2 associated genes in macrophages. Significant production of pro-inflammatory chemokine IL-8 was also observed. Seahorse and FLIM analysis revealed that cholesterol crystals drive metabolic reprogramming towards glycolysis with increased expression also observed of surrogate markers of glycolysis. Images of mitochondrial morphology reveal that cholesterol crystals promote significant mitochondrial fission. Finally, cholesterol crystal induced inflammatory responses were attenuated upon inhibition of glycolysis, highlighting the significant impact of metabolic reprogramming in macrophage phenotype and function. This study demonstrates for the first time that cholesterol crystals alter macrophage metabolism and drive M1 polarization in primary human macrophages.

Keywords: Atherosclerosis, inflammation, macrophage, immunometabolism, cardiovascular disease.

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The Burden of Post-Translational Modification (PTM)-Disrupting Mutations in the Tumor Matrisome

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In recent years, a wealth of "omics" data has enabled cancer research to better focus on the elements playing a major role in the tumor microenvironment (TME). The extracellular matrix (ECM) and the tumor "matrisome" - the ensemble of ECM and extracellular enzymes, growth factors and chemokines/cytokines - are of paramount importance [1,2]. While the importance of mutations in oncogenesis is well known, less is understood about the role of mutations occurring in extracellular proteins in cancer. In particular, altered post translational modifications (PTM) drive cancer development and metastasis through various mechanisms [3,4] but none is known about PTM-affecting mutations in the tumor matrisome.

In our study, 9075 tumor samples from 32 tumor types from The Cancer Genome Atlas (TCGA) Pan-Cancer cohort were analyzed. We identified 151088 non-silent mutations in the coding regions of the matrisome, of which 1811 affecting known sites of hydroxylation, phosphorylation, N- and O-glycosylation, acetylation, ubiquitylation, sumoylation and methylation PTM.

Surprisingly, while the matrisome has a higher frequency of non-silent mutations then the rest of the genome [5], PTM-disruptive mutations (PTM^{mut}) occur generally at lower frequency and show signs of a higher negative selection pressure on PTM^{mut} [6]. Also, 230 genes with PTM^{mut} are involved in reciprocal homo- and heterotypic interactions, 39 of which occur in a functional region as, e.g., in FN1, COL1A1, MMP2, ADAM10 and NTN4. We surmise that altering the function of those "hub" matrisome proteins could lead to major changes in the TME and may play a significant role in tumorigenesis [7]. Thus, understanding of PTM^{mut} could lead to translational applications to improve cancer treatment and outcome in future.

Keywords: extracellular matrix (ECM); matrisome; mutations; post-translational modifications, (PTM); pan-cancer

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Towards a renal tubulointerstitium in vitro model

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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, with fibrosis being the pathological endpoint of CKD, where an increase of ECM deposition is normally observed. [2,3] Therefore, 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 [4], which can offer viable alternatives to investigate underlying pathomechanisms and progression of kidney diseases. [5, 6] In this work, we used a new microfluidic bioprinter to manufacture polysaccharides (alginate and pectin) scaffolds, together with primary kidney cells isolated from a transgenic reporter mouse, showing keratin 8 conjugated with a yellow fluorescent protein (K8-YFP) (**Figure 1a**). Primary murine tubular epithelial cells (pmTECs), endothelial and fibroblast (pmFibroblasts) cells were successfully isolated, but further optimization is required for the culture and expansion of primary endothelial and fibroblast cells. pmTECs and pmFibroblasts were positive for cell-specific markers (**Figure 1b**). Therefore, endothelial cell line (HUVECs) and pmTECs were bioprinted, in a core-shell arrangement, mimicking the peritubular capillaries wrapping the renal tubule, showing high accuracy in cell deposition (**Figure 2a**) and the production of hollow filaments (**Figure 2b**).

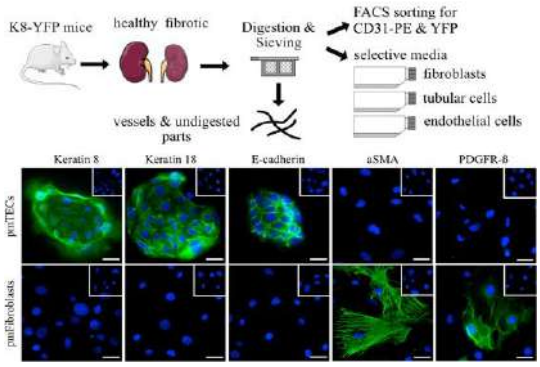

Figure 1: a) Isolation procedure for pmTECs, endothelial cells and pmFibroblasts from K8-YFP mice. b) pmFibroblasts and pmTECs were positive for cell-specific markers (inlays as negative control, scale bar=25

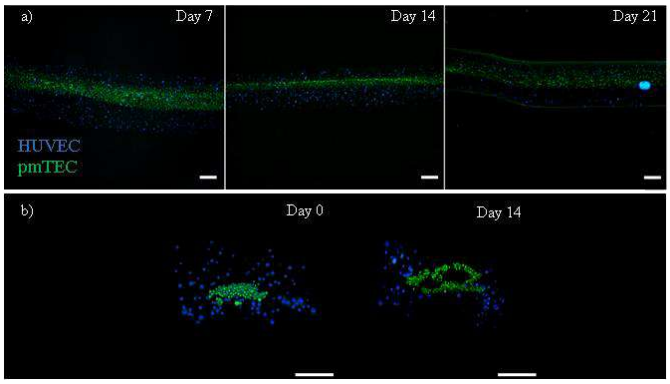

Figure 2: Core-shell bioprinted filaments made with blue-stained HUVEC as part of the shell and green-stained pmTEC as part of the core. a) Individual samples where imaged on day 7, 14 and 21. b) Confocal images of the core-shell filaments showing the formation of the hollow filament during the culture period, from day 0 (right after bioprinting) to day 14 (scale bar=200 µm).

This model will facilitate the investigation of the interstitial fibrosis, whose underlying mechanisms are currently not completely understood. [7] This study lays the basis for an alternative 3D *in vitro* model for the investigation of renal fibrosis mechanisms and its progression as well as a potential platform to screen novel therapies.

Keywords: Microfluidic, Bioprinting, Core-shell, Kidney, Renal 3D models, Polysaccharides

Acknowledgments: *We are grateful to the Dutch Kidney Foundation (Nierstichting Nederland, grant 18OI17 – Innovation Call 2018) and to the funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860715. We also acknowledge support from the Dutch Province of Limburg. This study was financially supported by the German Research Foundation (DFG: SFB/TRR57, SFB/TRR219, BO3755/3-1, BO3755/6-1 to PB and DJ100/1-1 to SD) and the German Ministry of Education and Research (BMBF: STOP-FSGS-01GM1901A) to PB and SD. Carmen. G. Tag (Division of Gastroenterology, RWTH Aachen, Germany) is acknowledged for FACS support.*

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Extracellular matrix tumorigenic alterations in prostate cancer organoids

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Organoids are small 3D cellular models analogous to their *in vivo* tissue of origin, realistically reproducing their architecture and function. For that reason, in last decades, they have been recognized as valuable pre-clinical models in the field of cancer research for the development of the so-called personalized therapy.[1,2]

Considering the incidence of prostate cancer (PCa) in men worldwide,[3] with the only effective treatment being invasive prostatectomy surgery, there is an urgent need to develop better *in vitro* models in order to disclose important tumor development and progression mechanisms and consequently develop more effective therapies. We have developed prostate cancer organoids (PCOs) by directly isolating the cells from primary tumor tissue after radical prostatectomy. We have confirmed, by immunohistochemistry, the multicellular composition of the PCOs, containing both epithelial and mesenchymal cells, as well as their invasive/migratory phenotype in 3D culture, resembling the *in vivo* tissue histological features.

It is well known that one of the hallmarks of adenocarcinomas is the increased extracellular matrix (ECM) deposition and stiffening, known as desmoplasia.[6] Although this has been demonstrated in 2D *in vitro* cell models, less is reported on the ECM remodeling dynamics in 3D tumor organoids. In particular, we are interested in studying the role of TGF-β in promoting tumorigenic alterations, by affecting the structural and functional changes in the ECM of primary PCOs. Our preliminary results, show that the TGF-β inhibition causes significant structural changes in the organoids morphology revealing well distinguishable luminal chambers, accompanied by a less invasive and migratory behavior of the PCOs. Additionally, the inhibition of TGF-β limits the deposition of ECM components as well as matrix remodeling capacity. Therefore, modulating TGF-β stimulation at the tumor primary location might help to diminish metastatic dissemination of PCa.

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YAP1 regulates size and contractility of cardiomyocytes

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YAP1 mechano-tranducer protein is ubiquitously expressed throughout various tissues, regulates organ size and regenerative processes. It has been shown previously that YAP1 expression in the cardiomyocytes is required for proper development and response to overload and myocardial injury. In response to myocardial injury YAP1 translocates into the nuclei of cardiomyocytes of infarction border zone.

We used in vitro model of human pluripotent stem cell cardiomyocytes to investigate, which modalities occurring in border zone of myocardial infarction promote YAP1 activation and what effect YAP1 activation exerts on cardiomyocyte function.

We mimicked increased cell strain and ECM remodeling in vitro to investigate role of YAP1 hypertrophic response of cardiomyocytes by using WT and YAP1 KO cardiomyocytes. Furthermore we used YAP1 gain of function approach to understand consequences of YAP1 activity on cardiomyocyte function in normal and DCM patient derived cardiomyocytes.

We conclude that YAP1 is required for hypertrophic response in cardiomyocytes and increases contractile force. Our data suggest that changes in the calcium metabolism contribute to increased contractile force induced by YAP1 activity.

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Calcineurin-NFAT signalling in human mesenchymal stromal cells drives ECM remodeling and anti-fungal response

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Mesenchymal stromal cells (MSCs) combined with calcineurin-NFAT (CN-NFAT) inhibitors are being tested as a treatment for graft versus host disease (GvHD). The immunosuppressive properties of MSCs seem beneficial; however, their response during fungal infection, which is a main cause of mortality in GvHD patients, is unknown. We report that MSCs phagocytose the fungal component zymosan, which results in phosphorylation of Syk kinase. The ensuing signalling cascade causes an increase in cytosolic calcium levels and ultimately, NFAT1 nuclear translocation. RNA-sequencing analysis of zymosan-treated MSCs showed that CN-NFAT inhibition affects extracellular matrix (ECM) genes, but not cytokine expression that is under the control of the NF-κB pathway. When co-culturing MSCs with human peripheral blood mononuclear cells, selective NFAT inhibition in MSCs decreased anti-fungal cytokine expression. These findings reveal a dual mechanism underlying the MSC response to zymosan: while NF-κB directly controls inflammatory cytokine expression, NFAT impacts immune-cell functions by regulating ECM genes.

Keywords: mesenchymal stromal cells; Calcineurin; NFAT; hyaluronan; extracellular matrix

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3D *In Vitro* Model of Early-Stage Human Cardiac Fibrosis Based on Bioartificial Scaffold

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Myocardial infarction causes cardiomyocytes loss, and extracellular matrix (ECM) remodelling, resulting in the progressive formation of a stiff dysfunctional scar mainly populated by cardiac fibroblasts[1]. Following the 3Rs principle, *in vitro* models of human cardiac post-infarct tissue could improve preclinical validation of new therapies aimed at heart regeneration. This work was aimed at the in vitro engineering of 3D human cardiac fibrotic tissue at its early stages of development. 3D square-meshed scaffolds with different pore sizes (150 µm; 350 µm) were fabricated from polycaprolactone (PCL) by melt-extrusion additive manufacturing. Then, they were surface functionalized with gelatin (G), exploiting mussel-inspired pre-coating, to support the long-term culture of adult human cardiac fibroblasts (AHCFs; PromoCell) and cardiac ECM production. Physicochemical characterisations were performed at each processing step. G grafting efficacy was confirmed by QCM-D, ATR-FTIR, static contact angle analysis that was used also to asses coating stability during 1 week incubation in phosphate buffered saline (PBS) at 37°C. After 3 weeks culture time, scaffolds were decellularized to evaluate by immunostaining the deposition of cardiac pathological ECM proteins such as Fibronectin, Laminin, Tenascin X, Collagen I-III-IV. AHCFs activation, triggered by scaffold stiffness, was demonstrated by the increase (compared to control) in α-smooth muscle actinin (α-SMA) and Discoidin Domain-containing Receptor 2 (DDR2) expression. Long-term culture of AHCFs on PCL-G square-meshed scaffolds allowed the engineering of human cardiac fibrotic

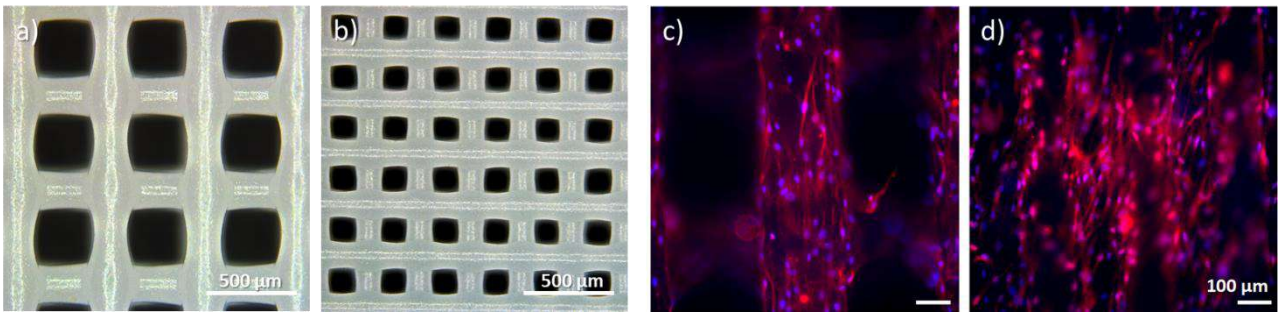

Figure 1 **– Scaffolds with larger (350 µm) a) and smaller (150 µm) pore size b). Fluorescence microscopy images of α-SMA expressed by AHCFs after 3 weeks culture on scaffolds with larger c) and smaller d) pore size.**

tissue, tuning cardiac ECM composition by scaffold pore size.

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In Vitro Engineered Model of Fibrotic Cardiac Tissue Based on Electrospun Bioartificial Scaffolds

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Myocardial infarction, the leading cause of mortality worldwide, provokes the massive loss of cardiomyocytes and the activation of a pathological remodelling, resulting in the overdeposition of highly crosslinked collagen by Myofibroblasts (MyoFs), myocardial fibrosis, ventricular 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* engineered human models reproducing post-infarct microenvironment for new therapy/drug screening are widely required.

In this work, an engineered *in vitro* model of human cardiac fibrotic tissue was designed in order to reproduce early-stage cardiac fibrotic tissue with low thickness. In detail, bioartificial scaffolds based on polycaprolactone (PCL) and coated with Gelatin (G) were fabricated. Randomly oriented nanofibrous PCL scaffolds, obtained by electrospinning, showed homogeneous fibers and small and interconnected porosities, closely mimicking the morphological features and architeture of native extracellular matrix (ECM). To further mimic the ECM chemical composition, PCL scaffolds were surface modified using a two-steps mussel-inspired strategy: G (Type A) was grafted exploiting an adhesive 3,4-Dihydroxy-D,L-phenylalanine (DOPA) precoating. After each functionalization step, physicochemical characterizations were performed to assess functionalization success and coating stability.

Human Cardiac Fibroblasts (HCFs) isolated from human ventricle were seeded on the biomimetic scaffolds at a density of $7x10^4$ cells/cm^2. Their adhesion, functional organization and biomatrix deposition were analysed at long-term culture times. In addition, intrinsic scaffold stiffness was exploited to promote MyoFs activation and proliferation.

Biological validation demonstrated bioartificial scaffolds capability to sustain HCFs adhesion and spreading. The expression of fibroblasts markers (α-SMA and DDR-2) and secretion of typical cardiac ECM proteins (Fibronectin, Laminin, Tenascin and Collagens) were confirmed by immunofluorescence analysis.

In conclusion, in this study an *in vitro* model of human cardiac fibrosis at its early stages was engineered as a promising platform for testing new regenerative therapies.

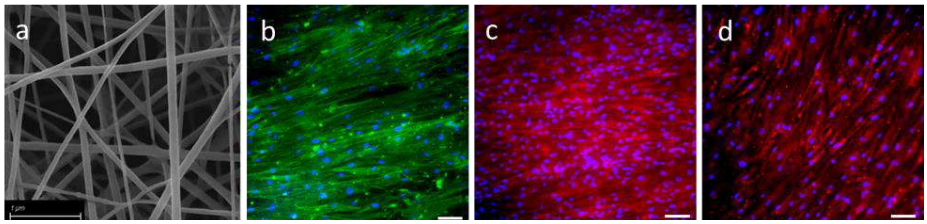

Figure 1 SEM image of electrospun PCL nanofibers; scale bar of 1 µm (a). Phalloidin staining for F-actin (b) and immunofluorescence images of expressed DDR2 (c) and α-SMA (d) on HCFs cultured for 3 weeks on electrospun scaffolds, bar

Acknowledgments: *This research was supported by BIORECAR project funded by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 772168).*

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The Effect of iPSC Culture Conditions Prior to Downstream Kidney Organoid Differentiation

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Nephrotoxicity screening is a crucial element of the safety-screening phase during drug development. Furthermore, drug-induced toxicity is a significant cause of acute kidney injury in patients worldwide. Therefore, it is essential for researchers to have access to advanced kidney models in order to study these adverse toxic effects. However, current models used for nephrotoxic screens do not recapitulate the intricate human renal cellular composition, microenvironment and function[1]. 2D *in vitro* cell models are not complex enough to mimic organ tissue form or function. *In vivo* animal models are also problematic as they significantly different to human physiology, as well as associated ethical issues and high costs. Recent establishment of protocols for generating stem cell derived kidney organoids has opened the door for the development of alternative complex kidney *in vitro* models[2,3,4]. However, there are still limitations to the production of reproducible kidney organoids at a large scale. In this study, we compared various conditions prior to differentiation, such as; culturing substrate, starting cell density and culture systems, and assess their downstream effect on kidney organoid productivity. Preliminary results suggest that culturing substrate and cell density have a significant impact on differentiation efficiency. The results also show comparable outcomes in terms of cell pluripotency levels for SSEA-3, SSEA-4, Tra 1-60 and Tra 1-81 markers as confirmed by flow cytometry, and immunofluorescence of SOX2 and OCT3/4. The extent of the impact of these parameters in the differentiation of cells in kidney organoids is currently under investigation.

Keywords: kidney, induced pluripotent stem cells, organoids.

Acknowledgments: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860715.*

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Generation and Characterization of Organotypic Cardiac Microtissues for Translational Research

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Cardiovascular diseases (CVD) remain as the leading cause of death worldwide, and there is an increasing demand to develop faithful models of human heart tissue for pre-clinical research. While recent technologies provide some insight into how human CVDs can be modelled *in vitro*, they may not always give a comprehensive overview of the complexity of the human heart due to their limits in cellular heterogeneity and physiological complexity [1] Furthermore, animal models may not always faithfully reflect the features that are unique to human biology and disease [2]. 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 better recapitulate the physiological complexity of the human heart.

We have optimized a scaffold-free protocol to generate multicellular, beating, self-organized and functional cardiac spheroids derived *in vitro* from hiPSCs – which can be also defined as "human cardiac organoids" (hCOs) [1], [3]. The hCOs 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. Ongoing prospects include RNA-seq time-course analysis of hCOs, compared to traditional monolayer differentiated hiPSCs and human heart tissues. Our results demonstrate that culture dimensionality is an important mediator of the cellular response to its biophysical environment, and able to modulate their survival, differentiation, and collective organization

Overall, our model could represent a powerful platform for future translational research in cardiovascular biology.

Keywords: cardiovascular biology, 3D-cell culture, stem cells, organoids

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Preclinical evaluation of autologous artificial connective tissue made with oral mucosa and skin fibroblasts to increase keratinized gingiva.

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The gingiva is a connective tissue that surrounds teeth and dental implants, forming a barrier that protects against periodontopathogens. Its loss leads to the accumulation of dental plaque and periodontal/peri-implant diseases[1]. Hence, various surgical procedures are used to replace lost tissue. Free gingiva and oral connective tissue autografts are the gold standard treatments[2]. Unfortunately, they are limited by the amount of autologous tissue available for grafting. For that reason, collagen scaffolds and artificial tissues have become an alternative source of tissue[3].

This work developed and characterized autologous oral and skin artificial connective tissue by seeding collagen I scaffolds with autologous oral fibroblast or skin fibroblasts. At the time of grafting the profile of proteins secreted by both tissues in the culture medium was determined. After creating a porcine model of keratinized gingiva reduction both tissues were grafted in contralateral wounds and clinical follow-ups and wound healing evaluation (profile of proteins in wound exudates and histological analysis of treated wounds) were carried out. The graft of artificial skin and oral connective tissue led to the formation of keratinized gingival tissue of the same color and texture as the healthy adjacent tissue. The width of the keratinized gingiva 30 days after surgery was clinically relevant (success ≥ 2mm). Mature fibroblasts and randomly oriented collagen fibers were observed in wounds grafted with artificial oral connective tissue, whereas unidirectional collagen fibers parallel to the wound surface were observed in wounds grafted with artificial skin connective tissue. In both cases, the thickness of the epithelium was similar to that of healthy tissue. A differential expression of protein factors was observed between both tissues before and after grafting, suggesting that they could differentially affect early repair events that influence the healing process.

Although the clinical results are similar, the histological differences between wounds grafted with oral or cutaneous artificial connective tissue and the differential secretion of protein factors that play a role in wound healing indicate that healing of wounds grafted with artificial oral connective tissue was better than that of wounds grafted with artificial skin connective tissue.

Keywords: keratinized gingiva, membrane, collagen, fibroblast, oral

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Environmental mechanical parameters influence deposition of ECM

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Introduction: To generate cartilage-like tissue *in vitro* or *in vivo*, biomaterial development plays a pivotal role in designing novel strategies augmenting the formation of the new tissue. Hydrogels are valuable platforms to study the interaction between cells and material properties while mimicking the natural micro-environment of the cell. In the field of tissue engineering, hyaluronan (HA) – based hydrogels received increasing attention in recent years due to their biocompatibility and bioactivity. Consequently, a class of HA composites was engineered to offer a tunable platform to study cell response to different environmental conditions[1]. Recent groundbreaking studies indicated that hydrogel mechanical properties (i.e. stiffness) can influence the spatiotemporal distribution of secreted extracellular matrix (ECM) components in the pericellular space[2] . In this study we investigate how specific environmental parameters of tunable HA gels (i.e. stiffness, crosslinking density) influence cellular behaviour and ECM deposition throughout the construct during chondrogenic differentiation of mesenchymal stromal cells (MSCs).

Material and Methods: Hyaluronan conjugated with tyramine was reconstituted overnight in PBS and crosslinked with Horse Radish Peroxidase and 150 µM, 300 µM or 600 µM hydrogen peroxidase (H_2O_2) to form hydrogels with different crosslinking degrees. The mechanical properties of the gels were measured by rheology (oscillatory shear tests, amplitude and time sweeps, stiffness G', G'') and the pore size was determined from the swelling ratios at equilibrium using the Flory-Rehner model. Chondrogenic differentiation of encapsulated human bone marrow derived mesenchymal stromal cells (BM-MSCs) was performed *in vitro* and samples were processed for rt-qPCR at day 14 and 28 and for histology at day 28.

Results: HA-tyr hydrogel stiffness increased and mesh size decreased with increasing H_2O_2 concentration. All hydrogel conditions supported cell viability and MSCs chondrogenic differentiation (expression of *COL2A1* and *ACAN*) with higher *COL2A1* expression with increasing crosslinking density. mRNA expression of matrix degrading enzymes (*MMP1*, *3*) was upregulated in the gels at 600 µM [H_2O_2]. Immunohistochemistry revealed a homogeneous distribution of type II collagen gels at 150 µM [H_2O_2], less distributed in the 300 while it was mainly localized in the pericellular space in the stiffer gel condition.

Conclusion: Our study indicate that mechanical/environmental parameters can influence ECM deposition and spatial translocation possibly via upregulation of ECM degrading enzymes. Understanding the underlying mechanism by which these events take place, could reveal novel concepts of mechanoregulation of cell behaviour that can be exploited to create functional materials that offer the optimal micro-environment for cells to form and regenerate tissue.

Keywords: hydrogels, mechanoregulation, MSCs, chondrogenesis

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Production of a microfluidic chip to study the CD44-Hippo pathway cross-talk

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During the last decade, demand for physiologically relevant 3D *in vitro* models has arisen since these models are capable to better recapitulate complex cell behaviour than 2D cell cultures as well as reduce the need for animal testing. However, commercially available chips usually have a rectangular cross-section of the channels, which may cause non-physiological behaviour of the cells due to disturbances in shear stress. It is now well established that laminar flow generating constant shear stress poses a protective effect on vasculature. On contrary, disturbed flow is responsible for the pathologies. Among many mechanosensitive pathways, the Hippo pathway was shown to sense the shear stress in endothelial cells[1]. CD44 is a promiscuous mechanoreceptor that senses the ECM components in addition to other functions. Literature has indicated that it may interact with the Hippo pathway[2].

The monitoring of the Hippo pathway was optimised in a static HUVEC cell culture. First, a fluorescence reporter of Hippo pathway-related transcription was introduced. It, however, worked well with confocal microscopy only. The expression of Hippo pathway downstream genes was studied by RT-qPCR, reference gene for HUVECs was optimised. Hippo pathway activation was further detected using western blotting. Next, The Hippo pathway activity will be examined under flow using our model with unique surface chemistry.

Acknowledgements:
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Tuning macrophage polarization to model myocardial infarction in the generation of functional cardiac organoids

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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 and immune response. 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.

METHODS: The first objective of this project is to obtain conditioned media obtained from immune cells for stimulating cardiomyocytes. In order to achieve this, iPSCs were differentiated to obtain macrophages (iMacs). Their expression of general macrophage (CD14, CD11b) and resident macrophage (CX3CR1, CCR2) markers were assessed, in addition to their phagocytic potential.

RESULTS: iMacs matured for 7 days with MCSF (Fig.1 A) were found to be 93.6% $CD14^{high}CD11b^{high}$ (Fig.1 B). Compared to blood-derived macrophages, CX3CR1 was upregulated (Fig.1 C) and CCR2 (Fig.1 D) was downregulated in iMacs showing a resident phenotype. Addtionally, iMacs also showed phagocytic potential (23.3%) (Fig.1 E,F).

DISCUSSION & CONCLUSIONS: Comprehensively, according to the preliminary data, iPSCs have been successfully differentiated into macrophages. However, they need to be characterized further to confirm their functional ability.

Keywords: iPSC, cardiomyocytes, macrophages, resident macrophages, engineered heart tissue.

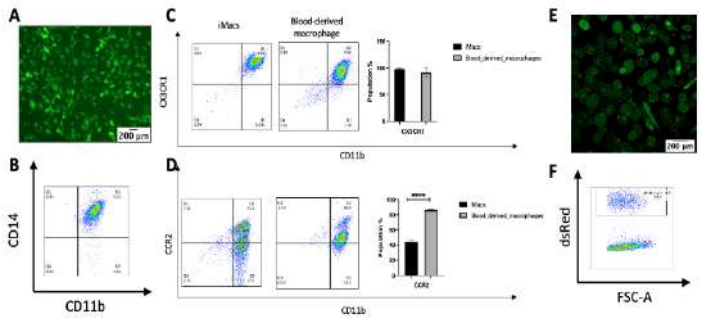

Figure 1 Characterization of iMacs. **A)** iMacs after 7 days of MCSF treatment, **B)** CD14 and CD11b expression of iMacs using flow cytometry, **C)** CX3CR1 expression of iMacs and blood-derived macrophages, **D)** CCR2 expression of iMacs and blood-derived macrophages, **E)** iMacs phagocytosing fluorescent red zymosan particles (40X), **F)** iMacs phagocytosis assay using flow cytometry. **** denotes p<0.0001, performed using t-test.

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Mechanobiological aspects of thrombolysis: *in vitro* study in vascular models

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Ischemic stroke is one of the most important causes of mortality and morbidity worldwide. Intravenous thrombolysis using recombinant tissue plasminogen activator (rt-PA) is primary and the most frequently used recanalization treatment method of acute ischemic stroke. Successful recanalization achieved by intravenous thrombolysis is, however, relatively low and the underlying mechanisms are not completely elucidated. In our long-term effort focused on uncovering particular mechanisms of thrombolysis using an *in vitro* models a set of biomechanical aspects was encountered. It includes clot structure, degree of retraction and compression.

Clots were prepared out of full blood or mixtures of purified fibrinogen with red blood cell by spontaneous or thrombin induced clotting. Static and flow *in vitro* models were used to study the rt-PA induced thrombolysis and/or recanalization.

A set of different clot types were prepared: red blood cell dominant, fibrin dominant, plasma clots and a range of semisynthetic clots with variable content of red blood cells. They were subjected to rt-PA induced thrombolysis in a static system. Data indicated that densely packed plasma clots and semisynthetic clots containing 2.5×10^9 red blood cells per ml were the most resistant to thrombolysis. Further the relation of degree of clot retraction and thrombolysis was evaluated in a static model. Red blood cell dominant clots were aged for 2 and 5 hours resulting in partly and well developed retraction. The overall lysis of well retracted clots was lower. The contribution of rt-PA induced lysis was, however, independent of clot age and corresponding retraction. The role of collateral circulation in thrombolysis and vessel recanalization was studied in a flow vascular model mimicking the occlusion of middle cerebral artery. The data recapitulated more rapid recanalization in presence of collateral circulation but the rt-PA induced thrombolysis was not affected. When trying to establish a clue for such controversy we found that collateral circulation significantly reduced clot compression at the site of occlusion.

Keywords: stroke, thrombolysis, rt-PA, clot

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Covalently cross-linked hyaluronic acid BASED hydrogels with tunable properties for cell culturing

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Cells can recognize various cues by extracellular matrix (ECM), starting from numerous chemical signals or proteins interactions to the mechanical properties of ECM itself. Each cell type favors a different set of these cues to work correctly. Therefore, a classical approach to in vitro cell culturing on plastic or glass surfaces comes with limitations as they do not mimic ECM. There is a growing need for a biocompatible scaffold for cell cultures with tunable mechanical properties and bioactivity in recent years.

In this study, we present covalently cross-linked hyaluronic acid hydrogels, --established cross-linking chemistry with the addition of biomolecules (serum albumin, gelatin, collagen) long-term stability with the biocompatibility of the hydrogel. The tunable mechanical properties allow to preparation of hydrogels of various elastic properties, numerically characterized as Young's Modulus (YM) in the range from 0.1 to 20 kPa. Mechanical properties, internal structure, layer thickness, swelling capacity, and hydrogel biocompatibility were characterized by Atomic Force Microscopy (AFM), Holographic Microscopy (HM), Confocal microscopy, and viability assay, respectively.

Young's Modulus and actin structure were compared when mouse embryonic fibroblasts were cultivated on hydrogels and standard cultivation surfaces such as glass, plastic, and 0.1% gelatine. Results showed that cells on hydrogels were significantly softer, than on the standard surfaces as a group. Morphologically, cells on hydrogels tended to grow into the gel as opposed to stiff surfaces. Moreover, cells on hydrogels showed rich actin structures and the presence of filopodia. Lastly, our experiments with HL-1 cardiomyocytes with HA--based hydrogels showed great potential in improving electric activity and increased cell monolayer formation.

This study demonstrated that our hyaluronan-based hydrogel system could be utilized as a better option for cell culture. In addition, our hydrogels system can also be functionalized with active molecules to further modulate cells *in vitro.*

Keywords: Hyaluronic acid, Hydrogels, Atomic Force Microscopy

We acknowledge CF Nanobiotechnology supported by MEYS CR (LM2018127).

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PSC mesoderm specification is controlled by YAP-TEAD1-driven cytoskeleton dynamics and intracellular tension

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The dynamic remodeling of actin cytoskeleton plays a critical role in many biological processes including cell migration, division and differentiation.

Mechanical signals arising from the extracellular matrix (ECM) are converted into biochemical cues and transmitted through the cytoskeleton to the nucleus, where they regulate the expression of mechanosensitive genes. One of the most studied mechanism, in adult cells, entails the force-induced shuttling of YAP/TAZ from the cytoplasm into the nucleus to activate focal adhesion (FA) gene transcription, thus contributing to FA-cytoskeleton stability and cell mechanical properties. Its regulation and role in pluripotent stem cells (PSCs) are poorly understood. Here, we show the sustained basal YAP-TEAD1 transcriptional activity in human PSCs is insensitive to cell density and colony area and dependent of AMOT inactivation. In fact, the activation of AMOT, by inhibition of Tankyrase enzyme, restored the YAP sensitivity to cell density. Moreover, we show that YAP-TEAD1 complex: 1) targets key genes encoding for proteins involved in cytoskeleton dynamics, 2) mediates PSC stiffening in response to substrate rigidity and 3) regulates the PSC mechanical properties during germ layers specification.

Finally, we show the sustained activation of YAP–TEAD1 transcriptional axis hinders PSC mesoderm specification by interfering with the fine tuning of cytoskeleton remodelling needed for the differentiation process [1].

Keywords: YAP-TEAD1 complex, pluripotent stem cell mechanics, F-actin cytoskeleton dynamics, mesoderm specification

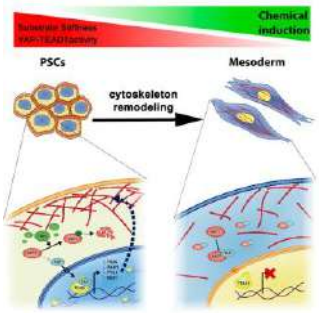

Figure 1: YAP-TEAD1 transcriptional activity in pluripotent stem cells (PSCs) is mechanically controlled by substrate stiffness to finely tune the transcription of a handful of genes involved in cytoskeleton remodeling during mesoderm specification. YAP shuttling to the nucleus during PSC specification is inhibited by AMOT.

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uHeart: A novel heart-on-chip coupled with uniaxial mechanical stimulation and an integrated electrical reading system for drug cardiotoxicity studies

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Current preclinical models don´t mimic human complex behaviour. Organs-on-chip (OoC), micro-engineered models replicating the functional units of human organs, have recently emerged as an innovative solution to provide preclinical tests with promising predictive power. This technology has the benefit to integrate mechano biological stimuli and sensors in the 3D biological system, emulating human physiological behaviour in a realistic way[1,2].

We present uHeart, a heart-on-chip able to provide mechanical stimulation by applying uniaxial cyclic strains on 3D cardiac microtissues. This organ-on-chip is also integrated with an electrophyisiological reading system capable to record electrical signal from the biological construct.

Neonatal rat cardiomyocytes or cocultured human induced pluripotent stem cells and human dermal fibroblasts were embedded in a fibrin gel laden at a concentration of 80-120 · 10^6 cells / mL and cultured on the heart-on-chip platform for 5-10 days with 10% uniaxial strain at 1Hz. Following this mechanical training, electrophysiological measurements were performed while cardiotoxic drugs were administrered in incremental doses.

Thanks to the mechanical training applied by uBeat technology, cardiac cells beat as a syncronicium after 7 days, promoting early spontaneous synchronous beating, superior cardiac differentiation, and higher contractile capability. Thanks to the integrated electrical reading system, electrophysiological signals of the constructs can be recorded *in situ* and several drugs were successfully tested to assess the efficacy of the develop model for drug cardiotoxicity assessment.

In conclusion, uHeart system responded to drugs in a physiological manner, appointing BiomimX OoC as a useful tool for preclinical drug cardiotoxicity studies.

Keywords: Heart-on-chip, Organ-on-chip, Mechanical stimulation, Microphysiological system, electrophysiological signal

Acknowledgments

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Toward the understanding of bio-nano interactions through mechanobiology

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Understanding cell-nanoparticle interactions has become one of the main objectives of Nanomedicine since its dawn (1). The physico-chemical properties able to improve nanomaterial's interaction with cells, and eventually enhance their internalization, have been extensively studied over the past decades (2-4). However, this huge effort, although significant, has failed to drive Nanomedicine toward a revolution in cancer treatment and, indeed, clinical translation of nanoparticle-based therapies is still poor (5). Therefore, a shift toward new paradigms in the interpretation of Bio-Nano interactions is desirable and the study of cell mechanic recently arose as a promising area of investigation (6).

In this regard, it was recently highlighted the possibility to target cancer cells or metastatic cancer cells considering their mechanical state, according to a process called Mechanotargeting (7). However, targeting molecular processes affecting cell's mechanics, in order to favourite a more efficient nanoparticle delivery, is still elusive and poorly investigated. Using nanoparticles with different size and coating we demonstrated that the inhibition of the key component of Hippo pathway able to regulate the cell's mechanics, i.e. yes-associated protein (YAP) (8), affects the internalization of the nanoparticles by the cancer cells, regardless their physical properties. Noteworthy, YAP inhibition has no effect on nanoparticles' uptake in healthy cells, in which its activity is less pronounced. Our study demonstrates that it would be possible to control the internalization of nanoparticles by controlling cells' mechanobiology, ultimately improving the specific targeting and delivery of nanotherapy.

Acknowledgments

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In-vitro model of the human blood vessel wall

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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.

Development of hybrid hydrogels for osteochondral regeneration

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Osteochondral defects are lesions that involve both articular cartilage and the underlying subchondral bone and can be caused by aging, diseases (eg osteoarthritis), and traumatic injuries. Due to its avascular nature and the lack of both abundant nutrient and progenitor cells cartilage has limited ability to self-repair. Currently, several clinical treatments are available such as marrow stimulation and autologous chondrocytes implantation, but none of them has achieved the complete healing of the osteochondral lesion [1]. Stem cells have been proposed as ideal candidates for cartilage repair since a large number of cells can be obtained from different sources, however, selectively promoting stem cell differentiation into appropriate cell lineages in situ is still challenging [2; 3]. An expanding field of research has demonstrated that mechanical cues from the environment, such as stiffness, could drive tissue formation and maturation, suggesting that the modulation of mechanical properties of the scaffold could guide stem cell differentiation either into cartilage or bone lineages [3]. In this study, we developed a hybrid collagen (Col) and alginate (AG) hydrogel with tenable stiffness by varying the concentration of the crosslinker. Ovine MSCs were encapsulated into hydrogels of different stiffness and calcein-AM and ethidium homodimer were used to study viability. To evaluate cell proliferation within the hydrogels, oMSCs were stained using CFSE CellTraker. Moreover, nutrients uptake was studied using a fluorescent glucose probe within the 3D culture. Lastly, cellular morphology of oMSCs within the hydrogels was assessed using Phalloidin staining after 7 days of culture. We managed to produce scaffolds with increasing stiffness by modifying formulation parameters. Live/dead and CFSE staining showed that cells were viable and proliferating within all formulations. All hydrogels assure good nutrients transport as demonstrated by glucose uptake. Interestingly oMSCs retained a rounded chondrocyte morphology within all formulation, but on softer substrates, the tendency to form aggregates was enhanced suggesting that the hydrogel itself, without any exogenous stimuli, might affect oMSCs commitment.

Keywords: cartilage regeneration, stiffness, stem cells

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Cellular mechanosensing by means of atomic force microscopy

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Mechanobiological sensing brings together biology, physics, medicine and engineering, thus helps to characterize how the protein molecules, cells and tissues respond to mechanical cues contribute to differentiation, development, structural and disease processes. The mechanobiology contributes to recognition of the sensing, transduction and application of mechanical signals by the biological systems. Atomic force microscopy (AFM) has grew up from the solid material characterization method to the a important device allowing the simultaneous topographical and mechanical characterization of living biological systems.

In this work such a potential of the AFM method will be described on selected examples. It was shown that cell stiffness determined by AFM can be used as a marker for cancer progression and metastatic potential. Different cancer types feature distinct cell stiffness and a connection between attenuated cell stiffness and increased invasion capacity was also observed. The force microscope can serve as mechanotransducing actuator of the cardiac cells contractility. Combination with the other methods, such as microelectrode array, leads to a comprehensive description of the contractile phenomenon. Pathophysiological electro-mechanical coupling needs to be characterized in a detail, if the alterations often resulting in mechanical heart failure would be understand and treated. \We would like to demonstrate AFM together with other biophysical merthods brings a promising approach that helps understand the correlation between the cell structure, cell mechanics, and function.

Keywords: mechanobiology, mechanosensing, cells, Atomic Force Microscopy

We acknowledge CF Nanobiotechnology of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127).

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Material fluidity promotes the senescence of breast cancer cells in a fluidity-dependent manner

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Breast tissue is known to behave as a viscoelastic tissue, by which comprises of viscous and elastic components. The effect of elastic components of matrix on the breast cancer cell behavior has been widely studied. However, despite the physiological relevance of matrix viscosity in breast cancer growth (e.g. malignant breast tumor was found to be more viscous than the benign ones); the role of matrix viscosity on regulating breast cancer behavior is still poorly understood. To enable us for understanding the role of matrix viscosity on the cellular behavior, a material with minimized interference of the elastic component is necessary.

A hydrophobic viscous-dominant cell culture platform, or so-called **fluidic material**, with different levels of fluidity has been developed based on the Poly(caprolactone-*co*-D,L-Lactide) [P(CL-*co*-DLLA)] [1]. The molecular weight of P(CL-*co*-DLLA) was manipulated to control the fluidity level of the fluidic material. In this study, fluidic substrates with different levels of fluidity (**low, moderate, high**) were prepared by a spin-coating method. Next, the non-invasive breast cancer cell line, MCF-7, was cultured at the interface of the fibronectin-coated fluidic substrates.

By using the fluidic substrate, the effect of elasticity could be completely decoupled from the viscosity effect, as the fluidic substrates behave as liquid-like (G”/G’>1). Fluidic substrates are also stable in water, owing to their hydrophobic nature (θ>90˚). The MCF-7 cells formed cellular aggregates on the fluidic substrates, as fluidity inhibits the force transmission via vinculin therefore the cells were unable to spread. Despite the hydrophobicity nature of fluidic substrates, the amount of the adsorbed protein (fibronectin) on the substrates is not significantly different from the glass substrate and fluidity level of substrate does not affect the amount of adsorbed fibronectin. Therefore, the aggregation is potentially promoted by the fluidity nature of material. Those cellular aggregates showed less proliferation ability compared to their counterparts grown on the glass substrate, but remained metabolically active overtime, which suspects that cells may undergo the senescence as reported in the previous study [2]. Cells cultured on the higher fluidity level of material expressed lower Ki67 marker which emphasizes the ability of breast cancer cells to sense the viscous element of material. Finally, those cellular aggregates showed higher intensity of SA β-Gal marker with increasing material fluidity. In addition, those cellular aggregates were confirmed to have accumulation of DNA damage marker, γH2AX, which separates the senescence from dormancy.

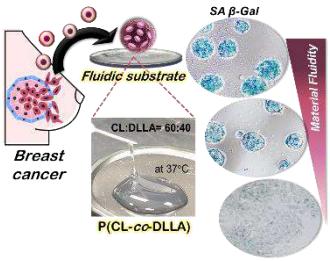

Figure 1. MCF-7 cells showed material fluidity level-dependent expression of senescence marker (SA β-Gal).

Keywords: material fluidity, breast cancer cells, spheroid, senescence

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Myeloperoxidase mediated alteration of endothelial function is dependent on its cationic charge

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Endothelial cell (EC) glycocalyx (GLX) comprise a multicomponent layer of proteoglycans and glycoproteins [1]. Alteration of its integrity contributes to chronic vascular inflammation and leads to the development of cardiovascular diseases. Myeloperoxidase (MPO), a highly abundant enzyme released by polymorphonuclear neutrophils, binds to the GLX and deleteriously affects vascular EC functions [2]. The focus of this study was to elucidate the mechanisms of MPO-mediated alteration of GLX molecules, and to unravel subsequent changes in endothelial integrity and function. MPO binding to GLX of human ECs and subsequent internalization was mediated by cell surface heparan sulfate chains. Moreover, interaction of MPO, which is carrying a cationic charge, with anionic glycosaminoglycans (GAGs) resulted in reduction of their relative charge. By means of micro-viscometry and atomic force microscopy, we disclosed that MPO can crosslink GAG chains. MPO-dependent modulation of GLX structure was further supported by alteration of wheat germ agglutinin staining. Increased expression of ICAM-1 documented endothelial cell activation by both catalytically active and also inactive MPO. Furthermore, MPO increased vascular permeability connected with reorganization of intracellular junctions, however, this was dependent on MPO's catalytic activity. Novel proteins interacting with MPO during transcytosis were identified by proteomic analysis. Altogether, these findings provide evidence that MPO through interaction with GAGs modulates overall charge of the GLX, causing modification of its structure and thus affecting EC function. Importantly, our results also suggest a number of proteins interacting with MPO that possess a variety of cellular localizations and functions [3].

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