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ABSTRACTS (BY NUMBER)

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Oral Presentations

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_198 - Confined bioprinting in inflatable bioreactor: toward the sterile bioproduction of tissues and organs

Christophe Marquette[1], Emma Petiot[1], Alexandre Dufour[1], Magali Barbaroux[2], Lucie Essayan[1], Celine Thomann[1]

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Introduction/Objectives
The future of organ and tissue biofabrication heavily depends on 3D bioprinting technologies[1] and their capacity to produce sterile, mature, functional, and physiological-sized tissues. Regardless of the bioprinting technology (laser, ink-jet, extrusion or volumetric bioprinting), maintaining sterility is a critical issue[2,3]. The only proposed solution to day is to bioprint in a biosafety cabinet where tissues are exposed to non-confined environment during transfer and culture medium exchanges. However, a biosafety cabinet is not sterile, but it is aseptic[4]. Sterility, as defined by the Centers of Disease Control and prevention, refers to "state of being free from all living microorganisms". Achieving sterility typically involves processes such as autoclaving, filtration, or irradiation to eliminate all microbial life. The present work describes an innovative component for the bioproduction of living tissue through bioprinting. The proposed technology is the missing link between the gold standard bioproduction in confined enclosures and the non-sterile state-of-the-art 3D bioprinting of living tissues.

Methods
We developed a bioproduction tool, the Flexible Unique Generator Unit (FUGU) system, enabling 3D bioprinting and culture in a confined and sterile environment. The concept is based on an inflatable and compliant bioreactor composed of a silicone membrane fixed to a building platform, itself hosting fluid management. This concept enables the full compliance during bioprinting, the modification of the internal culture volume, the handling of culture liquid phase, the direct observation and the gas exchange through the transparent silicone membrane.

Results
In the FUGU system, 3 features are essential: (i) being confined (ii) enabling 3D bioprinting (iii) allowing fluid transfer and management.

The system's ability to adjust internal volumes and ensure sterility, even under non-ideal conditions, is a testament to its robust design. The testing in various environments, from custom-built basic cartesian to sophisticated 6-axis robotic arm bioprinters, demonstrated its compatibility, flexibility and universality across different bioprinting platforms.

Then, confinement was evaluated with sterility assays through bacteria contamination and culture. Sterility, even in the presence of a direct bioprinting needle contamination, was obtained.

The FUGU's capacity to support bioprinting and culture of large tissues was demonstrated with a human cancer tissue model, opening up new possibilities for creating complex tissue structures. The successful cultivation of a 14.5 cm^3 tissue model, without external interventions, marks a significant milestone in the field.

Conclusions
FUGU sets a new standard in 3D bioprinting technology, offering a versatile, efficient, and sterile environment for biofabrication. Its ability to maintain sterility, adapt to various bioprinting settings, and facilitate the growth and development of large-scale tissues positions it as an invaluable tool in advancing tissue engineering, regenerative medicine, and beyond. The FUGU system represents a technological breakthrough and a step towards the future of personalised medicine and complex tissue replacement therapies.

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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_392 - Ion laden hydrogels as alternative bioinks for 3D bioprinting and ionic medicine

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Introduction/Objectives
The objective of this study is to develop ion-laden hydrogels, particularly based on alginate-di-aldehyde-gelatin (ADA-GEL), as candidate bioinks for three-dimensional (3D) bioprinting for skeletal muscle tissue engineering. Such ion-laden hydrogels are attractive in the emerging fields of ionic medicine [1]. By incorporating calcium-silicate based mesoporous bioactive glass nanoparticles (MBGNs) into the hydrogels, the aim is to enhance printability, material properties, and cellular behavior exploiting biological activity of ions and the mechanical support of the hydrogel matrix.
Methods: MBGNs were synthesized using the modified micro emulsion-assisted sol-gel method [2], [3]. The synthesized MBGNs were incorporated at different concentrations into ADA-GEL hydrogels to create ion-laden composite inks. The morphology, particle size, and structure of MBGNs were characterized, while the release of ions was analyzed to determine its impact on hydrogel crosslinking and the mechanical behavior of the scaffolds. Printability tests were conducted to assess the performance of the inks. Furthermore, C2C12 cells were encapsulated in the hydrogels and bioprinted to evaluate cell viability and morphology after the 3D bioprinting processes.
Results: Characterization of MBGNs revealed their spherical shape, amorphous nature, and particle size in the range 100-200 nm. The nanoparticles released silicon, calcium and dopant ions steadily over 7 days of in aqueous solution. ADA-GEL based inks showed suitable rheological properties, ensuring great printability. The incorporation of ion-releasing MBGNs into the hydrogels introduced an internal crosslinking process, which accelerated hydrogel gelation over time. Although this resulted in a shorter printable time window, the printability of the ink was enhanced. Filament fusion tests [4] indicated improved printing resolution, and filament collapse tests demonstrated ongoing crosslinking post-printing, leading to enhanced structural integrity. Bioprinting experiments with C2C12 cells showed improved cell viability in the ion-laden composite hydrogels compared to ADA-GEL without MBGNs, validating the beneficial effects of the bioactive ions on cell behavior.

Additionally, the printing process was optimized so that the bioprinted cells elongated toward the printing direction and aligned along the edge of the printed filaments.
Conclusions: Ion-laden ADA-GEL hydrogels incorporating ion-releasing MBGNs offer a promising alternative as bioinks for 3D bioprinting. The inclusion of MBGNs improves printability and promotes cell viability through controlled ion release. The ability to tune the properties and stability of these hydrogels via ion release demonstrates their significant potential for 3D bioprinting, opening up new possibilities in the field of regenerative medicine and ionic medicine.
Acknowledgement: The authors thank Deutsche Forschungsgemeinschaft (DFG); SFB/TRR225 (subproject B03) for financial support.
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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_656 - Achieving multidirectional fiber orientation in bioprinted constructs using submerged non-planar printing technology

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Introduction/Objectives
Native tissues often contain non-planar domains that contribute to the spatially varying orientation of extracellular matrix components and cells, affecting their biological structure and function. Therefore, the ability to mimic this complex arrangement of materials within 3D bioprinted constructs is a critical step towards the creating tissue models with more native-like structures. Traditional hydrogel-based bioprinting techniques typically use conventional slicing methods that are limited to layer-by-layer dispensing, which does not account for the flow-induced effects on natural fibrous hydrogels such as collagen. This study aims to combine non-planar slicing and submerged microextrusion bioprinting to control the orientation of fibers and cells in three dimensions within the same geometry.

Methods
Collagen-based bioinks were bioprinted under submerged conditions using both conventional and non-planar slicing for identical geometries. The rheological properties of the bioinks, including shear viscosity, storage, and loss moduli, were determined using a cone-and-plate rheometer. These parameters were then used in the numerical simulations of the bioprinting process to assess the pressures and stresses in the nozzle that can induce flow-induced alignment of collagen fibers and cells. After printing, the bioprinted constructs and control samples were cut and histologically stained to visualize the collagen fibers and cells at different points within the geometry. The stained samples were then analyzed to determine the overall cell and collagen fiber orientation relative to the local extrusion directions.

Results
Numerical simulations indicate that the intensity of the fiber orientation can be adjusted by specific input parameters, that directly affect the shear stress within the nozzle. Under submerged printing conditions, the printing speed can be reduced, allowing the shear stress to be applied for longer, to achieve the desired fiber orientation while maintaining high cell viability. Printed constructs showed up to 50% higher fiber orientation compared to the casted control. Unlike conventionally sliced samples which are limited to orientation in 2D planes, non-planar sliced samples achieved orientation in all directions.

Conclusions
The results demonstrate that submerged non-planar bioprinting can be used to precisely align fibers and cells in three dimensions, capturing the multidirectional orientation and curvature of functional tissues in nature. This approach has significant potential for the development of advanced tissue models that closely mimic native tissue structures.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_688 - Development of a bioprinted bilayer human endometrial model for studying embryo implantation

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Introduction/Objectives
Developing precise in vitro models of the human endometrium is essential to unravel its physiological and pathological roles, including its critical involvement in embryo implantation and recurrent implantation failure (RIF). Current models fail to capture the structural and functional intricacies of the endometrium, limiting their utility. This study introduces a novel bioprinted endometrial model comprising epithelial and stromal cells in a bilayer construct, designed to replicate the dynamic endometrial environment and support embryo implantation studies.

Methods
Human endometrial epithelial (RL95-2) and stromal (T HESCs) cell lines were used alongside JAR spheroids representing human blastocyst cells. Alginate-based bioink formulations, cross-linked with calcium chloride or calcium gluconate, were optimized for 3D cell bioprinting using the Allevi-3 printer. Key bioink parameters, including spreading ratio, printed line width standard deviation (SD), and degradation rates, were assessed. Cell viability was evaluated over 7 days using PrestoBlue assay. Fluorescent labeling (PKH67 for RL95-2, PKH26 for T HESCs) enabled imaging of bilayered constructs, which consisted of a stromal layer (T HESCs) and an epithelial layer (RL95-2) bioprinted into 4 mm disks. Hormone responsiveness was analyzed by sequential treatment with estradiol (proliferative phase) followed by estradiol and progesterone (secretory phase). Functional assays included Western blotting to measure E-cadherin expression and zymography for matrix metalloproteinase (MMP) secretion. JAR spheroids were applied to the epithelial layer to simulate embryo implantation, and interactions were visualized with confocal microscopy.

Results
The optimized bioink formulation (1.5% calcium gluconate) exhibited superior characteristics, including reduced spreading ratio, lower SD of printed line widths, slower degradation rates, and enhanced cell viability compared to other tested formulations. Fluorescent microscopy confirmed clear stratification of RL95-2 and T HESCs cells, mimicking the layered architecture of native endometrium. Sequential hormonal treatments revealed significant physiological changes in the constructs. Estradiol exposure enhanced cell viability by day 2 ($p < 0.05$), while progesterone treatment reduced cell viability by day 5 ($p < 0.05$), consistent with secretory phase adaptation. Hormone-treated constructs displayed significantly lower E-cadherin expression and reduced MMP-2 secretion after 5 days ($p < 0.05$), mirroring in vivo endometrial remodeling under progesterone influence. JAR spheroids adhered to and infiltrated the epithelial layer, effectively simulating embryo implantation.

Conclusions
This bioprinted system represents a significant advancement in reproductive biology, providing a robust platform for in vitro investigations of endometrial receptivity and implantation. With its biomimetic design, hormone responsiveness, and capacity to support embryo attachment, the model holds great promise for uncovering the mechanisms of implantation and addressing recurrent implantation failure (RIF). Its innovative features pave the way for deeper

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insights into endometrial dynamics and the development of personalized therapeutic approaches in reproductive medicine.

Topic: Submit to SYMPOSIUM
Subtopic: Biophysical Therapies for Tissue Regeneration
Type: Oral presentation

TERMIS25_1386 - Magnetically responsive 3D bioprinted constructs for enhanced tenogenic differentiation of adipose-derived stem cells

Vittoria Altheman[1], Giacomo Cortella[2], Rosa Monteiro[3], Alberto Pardo[4], Silvia Chiera[4], Manuel Gomez-Florit[4], Ana Liz Alves[5], Rui Domingues[3], Manuela Gomes[6]

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Introduction/Objectives
Tendon diseases are a leading cause of decreased performance and early retirement from sports in both humans and horses. Despite advancements in therapeutic approaches, achieving satisfactory tendon repair remains challenging. This study aims to determine whether external magnetic stimuli can enhance the tenogenic differentiation potential of stem cells embedded within a dECM-based 3D bioprinted magnetically responsive construct.

Methods
Tendon-mimetic constructs were fabricated through 3D bioprinting using decellularized extracellular matrix (dECM) bioinks derived from equine and porcine tendons, designed to replicate tendon microenvironments composition. Adipose-derived stem cells (ASCs) were incorporated into the dECM bioinks incorporating magnetic nanoparticles, which were anisotropically aligned within the scaffolds using an external magnetic field. Bioprinted constructs were then cultured with and without magnetic field exposure to assess the impact of magnetic stimulation on the expression of key tenogenic markers at both the protein and gene levels, as well as on the production of tendon-like extracellular matrix.

Results
Our findings indicate that dECM-based bioinks from both equine and porcine tendons can be successfully 3D bioprinted to produce biocompatible scaffolds that support ASC viability and promote tenogenic differentiation. Constructs exposed to magneto-mechanical stimulation exhibited upregulated expression of tenogenic markers and increased production of tendon-specific extracellular matrix proteins, compared to constructs cultured without magnetic stimulation.

Conclusions
This study demonstrates that combining biophysical cues (anisotropic alignment) with biochemical signals (tendon dECM composition) and remote magnetic stimulation can effectively promote tenogenic differentiation in 3D bioprinted tendon-mimetic constructs. These findings suggest that our system can replicate critical aspects of the tendon microenvironment, offering a promising strategy for tendon injury treatment.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_1393 - Post-operative anti-adhesive 3D printed chitosan membranes loaded with eugenol

Simona Braccini[1], Cinzia Guglielmi[1], Elia Pagliarini[2], Barbara Tarparelli[1], Emma Soddu[1], Diana Di Gioia[2], Grazia Totaro[1], Antonella Battisti[3], Sangram Keshari Samal[4], Dario Puppi[1]

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Introduction/Objectives
Postoperative adhesions represent a critical complication that impacts up to 95% of patients. The clinical consequences may include organ dysfunction and persistent discomfort requiring further surgery to remove scar tissue [1]. Anti-adhesion barriers are surgically employed to minimize contact between the surgical site and surrounding tissues, thereby preventing the formation of fibrous bands. The main objective of this work was the preparation of anti-adhesive chitosan membranes loaded with eugenol, a natural compound with antioxidant, antimicrobial, and anti-inflammatory properties [2], exploitable for the prevention and treatment of post-surgical adherences.

Methods
Chitosan membranes incorporating an eugenol emulsion (CS_Eug) or eugenol-loaded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles (NPs) (CS_NPs_Eug), were fabricated by means of 3D printing [3]. Membranes and NPs encapsulation efficiency (*EE*) and loading capacity (*L*) were analyzed using UV-Vis spectrophotometry. The antioxidant activity of the designed systems was studied with a radical scavenging assay and an enzymatic biodegradation test of the polymeric matrix was performed. Membranes biocompatibility and anti-adhesive properties were assessed through *in vitro* cell culture of the murine fibroblast cell line Balb/3T3 clone A31. Membranes antimicrobial activity was also evaluated against *E.coli* and *S.aureus*. All analyses were performed in triplicate and data processed by one-way ANOVA or t-student test.

Results
Optimized 3D printing protocols for the fabrication of two different kinds of eugenol-loaded chitosan membranes, i.e., CS_Eug and CS_NPs_Eug, were developed. The different fabrication strategies as well as processing conditions tuning allowed the obtainment of statistically different values of *EE* in the range 53–58 %, *L* in the range 1–9 %, and antioxidant activity ranging from 18–23 %. Evaluation of *in vitro* lysozyme biodegradation showed a membrane weight loss of 52–61% after 42 days of treatment. Furthermore, the designed devices were cytocompatible, prevented significant cell adhesion, and had strong antimicrobial activity.

Conclusions
The proposed 3D printing approach is suitable for the fabrication of customizable chitosan membranes loaded with eugenol. Membranes biocompatibility, anti-adhesive behavior, antioxidant activity, and antimicrobial properties were demonstrated *in vitro*. Furthermore, their biodegradation time was suitable to control the critical phases of the tissue healing process, as required to prevent the formation of post-operative adhesions.

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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1520 - Hybrid microfluidic-assisted 3D bioprinting and a new frontier for the assembly of multi-tissue models

Gianluca Cidonio[1], Salvatore D'alessandro[1], Sajad Mohammadi[1], Lucia Iafrate[1], Marco Bastioli[2], Biagio Palmisano[1], Mara Riminucci[1], Giancarlo Ruocco[2]

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Introduction/Objectives
3D bioprinting has revolutionized tissue engineering by enabling the fabrication of complex, biomimetic constructs. However, challenges such as limited heterogeneity and poor spatiotemporal control over bioactive factor release hinder clinical translation. This study presents an innovative hybrid 3D microfluidic-assisted bioprinting approach harnessing nanoclay-based bioinks for the compartimentalisation of functional biologics for cancer modelling as well as skeletal tissue regeneration. Specifically, the objectives of this study are (i) to enhance printability and controlled release of therapeutic agents, (ii) to engineer a new paradigm for 3D bioprinting and drug compartimentalisation, (iii) to develop cancer and skeletal tissue models for therapeutic testing.

Methods
A Microfluidic-Assisted Open-Source 3D Bioprinting System (MOS3S) was utilized to fabricate constructs using nanoclay-enhanced bioinks. Material inks designed based on combination of alginate, gelatin methacryloyl (GelMA), and nanoclay (Laponite®) were prepared and physically characterized to optimize printability and stability. Controlled drug release and cellular encapsulation were achieved using coaxial extrusion and high-throughput dispensing. Anti-cancer (Doxorubicin) and osteogenic (BMP-2) agents were incorporated via MOS3S high-throughput dispensing mode into 3D scaffolds for cancer and skeletal tissue models, respectively. Cell viability, proliferation, and differentiation were assessed using confocal imaging, viability assays, and gene expression analyses. Chick chorioallantoic membrane model was carried to evaluate successful vascular infiltration following pro-angiogenic factors dispensing.

Results
The inclusion of nanoclay into bioinks improved structural integrity, rheological properties, and sustained drug release. The use of high-throughput patterning of drug-loaded nanocomposite droplets was validated with the dispensing in various 96-well plate designs. High-throughput dispensing successfully localized bioactive agents, achieving reproducible and precise scaffold geometries. Cancer models demonstrated effective compartmentalization of Doxorubicin, with sustained drug release leading to reduced cancer cell viability (8% at Day 7) compared to untreated scaffolds. Skeletal tissue models showed enhanced osteogenic differentiation, evidenced by increased expression of RUNX2, ALP, and OCN genes. Thus, drug release profiles from nanoclay-based scaffolds exhibited a controlled and prolonged release compared to conventional materials, ensuring localized therapeutic delivery.

Conclusions
The hybrid 3D microfluidic-assisted bioprinting approach addresses key limitations in current bioprinting technologies by combining enhanced material performance with high-throughput bioactive agent delivery. This platform demonstrates potential for engineering functional cancer and skeletal tissue models, paving the way for improved drug testing and regenerative therapies. The sustained release of therapeutic agents coupled with precise structural fabrication provides a robust framework for advancing applications in regenerative medicine and TERM approaches.

Topic: Tissue-Specific Focus
Subtopic: Lung
Type: Oral presentation

TERMIS25_100 - Development of a primary cell-based respiratory epithelium model using dual 3D bioprinting technologies

Albane Carré[1 2 3], Celine Thomann[1], Alexandra Erny[2], Lucie Essayan[1], Karen Moreau[3], Fabienne Archer[2], Emma Petiot[1]

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Introduction/Objectives Respiratory infections remain the fifth leading cause of death globally, despite advances in risk reduction and treatment development. With the desire to reduce animal model use, *in vitro* human lung models have emerged, where the current gold standard is a simplistic 2D monolayer of cells cultured at air-liquid interface (ALI). Few existing strategies provide compartmentalisation and cm^2-scale models that mimic native lung tissue architecture and functions. Among them, 3D bioprinting stands out as one of the most advanced strategies for producing complete tissue models in a semi-automated fashion. We aimed to leverage two bioprinting technologies to create a complex 3D bronchial model with a primary cell-based basal compartment and mature epithelial layer.
Methods Micro extrusion printed primary lung fibroblasts (NHLF; 0.5 Mcells/mL) and human vein endothelial cells (HUVEC; 2 Mcells/mL) encapsulated in alginate, gelatin, and fibrinogen bioink, using a BioAssembly Bot 6-axis arm. This process yielded cm^2-scale models, with tuneable macro-geometry and a thickness under 1mm. After two weeks of maturation, human bronchial primary epithelial cells (hAEC) or cell line (Calu-3) were top-seeded either manually or by ink-jet using a Scienion SciFLEXARRAYER S3. Models were placed at ALI 2 to 3 weeks after epithelial seeding and maintained for 2 to 3 more weeks. Media screening was conducted to select the best conditions for the tri-culture. For hAEC, optimal seeding densities and duration of the two following maturation phases were tested to pursue pseudostratification and differentiation.

Throughout the models' culture, viability and metabolic activity were assessed by Live/Dead staining and lactate quantification respectively. The tissue histology was characterised by haematoxylin and eosin colouration weekly. NHLF and HUVEC were specifically identified using Collagen I and EN4 immunohistochemistry staining. Mucus production was assessed via Muc5ac staining. hAEC were identified via pan-cytokeratin staining and observed in transmitted electron microscopy.
Results First, we demonstrated the feasibility of printing lung primary cells using micro-extrusion to obtain a respiratory basal compartment. Our biofabricated model showed homogenous cell distribution, stable cell viability and increasing metabolic activity throughout the 14-days immersed culture period. Lung fibroblasts secreted a collagen lining on the model surface, supporting the following epithelial cell attachment.

Calu-3 cell inkjet seeding was achieved at a maximal cell density of 35 $kcells/cm^2$. Primary epithelial cell seeding demonstrated full surface coverage with manual seeding densities ranging from 30 to 120 $kcells/cm^2$. The intermediary density of 60 $kcells/cm^2$ was selected. Epithelial pseudostratified monolayer formation was confirmed for hAEC and Calu-3 before placing the models at ALI. Once at ALI, Calu-3 adopted a polarised organisation and mucus production was evidenced for manually top-seeded model. hAEC model demonstrated confluent epithelial monolayer with specific features such as microvilli and tight junctions but is still to be optimised to obtain differentiated cells.
Conclusions Future analysis will include protein quantification of the mucus by ELISA, qualification of the hAEC differentiation on the model throughout the maturation by fluorescent staining and the fine optimisation of the epithelial cell ink-jet deposition.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_122 - Bioprinted 3D pancreatic ductal tubes: a novel in vitro model for studying ductal cell maturation and disease progression

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Introduction/Objectives This study aims to develop a novel 3D in vitro model of pancreatic ductal tubes using advanced bioprinting techniques. We investigate the impact of geometry and spatial organization on the maturity, polarity, and functionality of pancreatic ductal cells. By creating hollow structures with precise cell positioning, we closely mimic the native architecture of pancreatic ducts, surpassing the limitations of traditional organoid cultures. This model will serve as a platform for understanding pancreatic duct biology, and investigating pancreatic plasticity, dysplasia, and cancer formation, with potential applications in studying genetic disorders affecting ductal tubes.
Methods We established a bioprinting protocol using cell suspensions printed into a solidified Ultimatrix substrate. We applied this technique to pancreatic progenitor (PP) cells derived from human pluripotent stem cells and differentiated the printed tubes into ductal cells using the differentiation protocol developed at our institute. Comparative analyses between bioprinted ductal tubes and patient-derived organoids (PDLOs) were conducted to evaluate the influence of spatial configuration on ductal cell maturation and functionality. To explore the model's potential for disease modeling, we investigated the effects of oncogenic mutations, specifically KRAS and GNAS, on ductal cell differentiation and tube formation.
Results Primary experiments using PP cells demonstrated successful formation of hollow tube-like structures. The bioprinted structures showed enhanced maturation and polarization compared to traditional organoids. Key markers of ductal cell identity including MUC1 and CFTR were more prominently expressed in the bioprinted tubes. Comparative analysis with PDLOs revealed that the bioprinted ductal tubes more accurately recapitulated the spatial organization and cellular arrangement of native pancreatic ducts. This was evidenced by improved lumen formation, cell polarity, and expression of functional markers. In our investigation of oncogenic mutations, we observed distinct phenotypic responses in the bioprinted model. KRAS and GNAS mutations elicited changes in cell proliferation, differentiation, and tube morphology that were more pronounced and physiologically relevant compared to 2D or organoid cultures.
Conclusions Our innovative bioprinted 3D pancreatic ductal tube model surpasses the limitations of traditional organoid cultures by closely mimicking the native architecture of pancreatic ducts. The enhanced maturation, polarization, and functionality observed in the bioprinted structures demonstrate the importance of spatial organization in recapitulating tissue-specific characteristics. Incorporating oncogenic mutations highlights its potential for studying the early stages of pancreatic cancer. Moreover, the model has potential applications in studying genetic disorders affecting ductal tubes, such as cystic fibrosis and Alagille syndrome. Providing a more physiologically relevant in vitro environment facilitates the targeted therapeutic strategies for various pancreatic disorders. Our findings demonstrate the potential of bioprinted 3D pancreatic ductal tubes as a powerful tool for understanding disease mechanisms and advancing personalized medicine approaches in pancreatic research, and potentially the development of novel diagnostic and therapeutic interventions.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_777 - Volumetric bioprinting of a vascularized and perfusable 3D in vitro model to recapitulate the exocrine pancreatic unit

Viola Sgarminato [1], Jorge Madrid-Wolff[2], Felix Wechsler[1], Christophe Moser[1]

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Objectives
The exocrine unit of the pancreas is the site where the earliest lesions of pancreatic cancer typically develop, making it a critical focus for developing efficient in vitro models for pancreatic cancer research. However, existing models often fall short in accurately replicating the complex 3D microanatomy of the exocrine unit. In this study, we provide a major innovation in the field of in vitro modeling by leveraging volumetric bioprinting (VBP) to fabricate a perfusable, vascularized model of the exocrine pancreatic unit in a single step and in square chamber that further enables microscopy imaging through the flat interface. This groundbreaking platform is designed to set a new standard in physiological relevance, enabling unprecedented insights into the early stages of pancreatic cancer and transforming the evaluation of potential therapeutic strategies.

Methods
Human fibroblasts (HFF-1) were embedded in a gelatin methacrylated (GelMA) solution containing a photoinitiator. A complex cellularized multiacinar cavity was fabricated using VBP in the square chamber for the first time. The VBP process was further refined to directly print perfusable channels within the chamber, featuring multiple inlets and outlets connected to lateral channels. Then human umbilical vein endothelial cells (HUVECs) were seeded into these channels, and blood vessel formation within the constructs was monitored.

Human pancreatic ductal epithelial cells (HPDE), stably expressing KRAS (HPDE-KRAS) were seeded within the multiacinar cavity through specific perfusable outlets. The ability of HPDE cells to adhere to and cover the cavity walls was analyzed using immunofluorescence imaging.

The viability of stromal cells within the GelMA constructs was assessed using Live/Dead and metabolic activity assays. Tumor-stroma crosstalk was evaluated over time by monitoring fibroblast activation through immunostaining and quantifying inflammatory cytokine release via ELISA.

Results
Complex structures mimicking the tubuloacinar gland morphology of the physiological exocrine pancreatic unit were successfully fabricated. These constructs provided a supportive environment for HFF-1, which remained viable and active for at least two weeks post-fabrication. HFF-1 cells formed a highly biomimetic network surrounding the 3D acinar cavities, closely resembling native cell organization.

The ability of HPDE cells to populate the constructs was demonstrated by their formation of a concave epithelial layer along the inner surfaces of the bioprinted structures. Under co-culture conditions, HFF-1 in contact with HPDE-KRAS cells exhibited a higher activation compared to HFF-1 co-cultured with healthy epithelial cells. We also proved the effectiveness of our biofabrication approach in fabricating perfusable vasculature in this model.

Conclusions
This perfusable fabricated 3D in vitro model is the first to recapitulate the tumor-stroma interactions observed in early-stage pancreatic cancer while accurately reproducing the anatomical structure of the exocrine gland. The rapid biofabrication process enables the production of multiple, scalable human models, making it suitable for high-throughput screening applications. Additionally, the ability to directly bioprint within perfusable chambers enhances the model's physiological relevance, allowing for the incorporation of functional vasculature and improving its potential for testing therapeutic strategies.

Topic: Tissue-Specific Focus
Subtopic: Pancreas
Type: Oral presentation

TERMIS25_956 - Ultrafast volumetric bioprinting of an endocrine pancreas using functional human iPSC-derived islets

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Introduction/Objectives
Generating functional islets from human induced pluripotent stem cells (iPSC) could unlock an unlimited cell source to tissue engineer an endocrine pancreas. Despite significant progresses in the differentiation protocols, current cell cultures cannot replicate intricate islet-environment interactions. This absence of reliable, native tissue-like environments limits the progress on pancreatic regenerative medicine, and in the development of new in vitro models to understand islet biology. Bioprinting technologies, biomaterials, and (stem) cell engineering provide a novel technological toolbox to pave the way towards the design of bioinspired systems that recapitulate salient organ functions. This study combines the generation of iPSC-derived islets with ultrafast volumetric light-based bioprinting to engineer an advanced pancreatic endocrine construct for modelling and regenerative medicine.

Methods
Human pancreatic islets were generated from iPSC following a seven-stage protocol. When reaching stage 7, iPSC-derived islets were collected and pancreatic constructs were generated via layerless and shear stress-free, light-based Volumetric Bioprinting (VBP). Concentrations ranging from 4%-7% (w/v%) of optically tuned gelatin methacrylate (GelMa) were used for bioprinting. iPSC-derived islets were characterized via staining and single cell transcriptomics. Insulin release on bioprinted constructs upon glucose exposure was assessed with static and dynamic glucose stimulated insulin secretion (GSIS). Bioprinted constructs were implanted in immunodeficient Balb/c NSG RIP-DTR mice and circulating human insulin levels measured over 3 months.

Results
VBP allowed to produce 50-60 mm^3 pancreatic constructs in 30 s and posed no mechanical or chemical stress on iPSC-derived islets, which were highly viable for 21 days after printing. Islet metabolic activity remained constant over time during 21 days of culture. Volumetric bioprinted iPSC-derived islets showed mature, insulin, glucagon and somatostatin, single-hormone producing cells after 21 days both characterized via staining and single cell transcriptomics. Bioprinted islets required a four day adaptation time after printing to recover their glucose-responsive insulin secretion profile both in static and in perfusion systems. Mathematically defined gyroids were bioprinted with a strut size of 750 µm and infill density of 33% considering the diffusion profile of human insulin to perform dynamic culture and GSIS. The system is compatible with supplementation with anti-diabetic drugs (i.e. liraglutide) for drug screening. Implanted mice survived surgical implantation of bioprinted constructs and experiments are currently ongoing.

Conclusions
Overall, VBP allows to successfully and viably bioprint iPSC-derived islets into geometrically defined morphologies while retaining their functionality. This technology opens up to new possibilities on developing novel tissue engineered platforms for modelling and therapy.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1318 - Embedded bioprinting of airway and alveolar stem cells to recapitulate native epithelial architecture

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Objective
The growing prevalence of chronic respiratory disease presents a significant global health challenge. While existing *in vitro* models, such as lung organoids and lung-on-chip devices, offer valuable insights into lung disease and treatment, they are unable to fully recapitulate the intricate architecture of the native lung. Recent advances in tissue engineering have made it possible to guide epithelial tissue morphogenesis by precisely controlling the deposition of stem cells within a hydrogel, as demonstrated in models of the colon, stomach, and intestine (*1*). The primary objective of this study was to extend these advancements to the lung by bioprinting airway and alveolar stem cells, while engineering more complex tissues that mimic the native branching architecture of the lung.

Methods
A novel basement membrane (BM)-mimetic hydrogel formulation was developed and utilized as the support bath for embedded bioprinting of airway and alveolar stem cells. This optimal hydrogel formulation was selected based on its ability to maintain the structural integrity of the printed cellular construct while promoting tissue formation and epithelial differentiation. High-density single-cell suspension bioinks were prepared from primary human bronchial epithelial cells and human induced pluripotent stem cell-derived alveolar type II cells for bioprinting airway and alveolar tissues, respectively (*2, 3*). The bioprinted constructs were maintained within the hydrogel support bath throughout the culture period, and maturation protocols were optimized to support lumen formation and epithelial differentiation. Histological analysis was performed on branching geometries relevant to the native architecture of the lung.

Results
Rheological characterization of the BM-mimetic hydrogel demonstrated appropriate stress yielding and shear recovery behavior, validating its suitability as a support bath for embedded bioprinting. Histological analysis of the airway tissues revealed the formation of an interconnected lumen across the branched structure within one week of culture, as indicated by E-cadherin staining. Similarly, the bioprinted alveolar tissues developed a single lumen within three weeks. The airway tissues, cultured in differentiation media for up to three weeks, produced robust pseudostratified epithelium with native-like lineage composition as indicated by the expression of Acetylated Alpha-Tubulin, Mucin 5AC, TP63, and Club Cell Secretory Protein. Meanwhile, the alveolar tissues maintained the expression of NKX2.1 and SFTPC.

Conclusions
This study reports a novel strategy and hydrogel support bath formulation for embedded bioprinting and subsequent maturation of airway and alveolar tissues, resulting in lung tissues with pre-defined geometry and interconnected lumen formation. These findings mark a significant step forward in engineering lung tissues that recapitulate the cellular composition and architecture of the native lung, providing a platform to enhance mechanistic understanding of lung development and pathogenesis, while laying the groundwork for producing implantable lung grafts.

Funding
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Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1470 - Generation and characterization of a complete urethral substitute by tissue engineering

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Introduction/Objectives
The urethra is a conduit responsible for the transport of urine from the bladder to the outside. It is composed of 3 differentiated layers: a tunica mucosa (TM), with a transitional or stratified epithelium,

which prevents the passage of toxic substances into the stroma; a highly vascularized corpus spongiosum (CE); and a tunica albuginea (TA), with contractile capacity. These layers are susceptible to different pathologies that can cause stenosis or dysfunctionality (1). Dorsal oral mucosal grafting is currently the gold-standard treatment for treating urethral strictures. To avoid problems associated with grafting, we have generated and characterized a full-thickness artificial urethral substitute by combining a substitute of each urethral layer (TM, CE and TA) generated with hydrogels and the main of cell types of these layers (2).

Methods
To generate the three urethral layers, two types of hydrogels were employed (fibrin-agarose for TM and TA, and fibrin for CE). Each hydrogel was generated with their corresponding human cell types: in TM layer, fibroblasts were encapsulated within the hydrogel and urothelial cells were cultured on the surface; in the EC, microvascular cells were used; in the AT, leiomyocytes were cultured. Artificials constructs were evaluated during 14 days of in vitro development in terms of cell viability, expression of specific molecules and biomechanical properties. Then, the three structures were subjected to plastic compression nanostructuration to generate a three-layered structure containing TM, CE and TA able to resemble the three main layers of the human urethra.

Results
Live and Dead results showed a high viability profile at all times and experimental groups. Moreover, immunohistological methods revealed the expression of markers characteristic of each layer. In short, the TM epithelum revealed multiple strata, with apical positive reaction to uroplakin-1. Microvascular cells of CE were positive to CD31 endothelial marker, whereas leiomyocites showed positive reaction against smooth muscle actin antibody. Finally, the biomechanical tests revealed improved mechanical properties of the multilayered urethral substitutes as compared to individual hydrogels, being more similar to native urethra.

Conclusions
The results of this work demonstrated the feasibility of generating a complete urethral substitute using tissue engineering nanostructuration methods. This artificial urethra was biomimetic to the native urethra, and shared important histomorphological and molecular characteristics of the native tissue, although further analysis is needed to determine its functional behavior.
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Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_614 - Combined use of olfactory mucosal mesenchymal stem cells secretome and neural guide tubes to promote peripheral nerve regeneration – sheep model assay

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Introduction/Objectives
Peripheral Nerve Injuries (PNI) are a frequent occurrence in modern societies, affecting both humans and animals. Despite the efforts and advances made in recent years, it has not yet been possible to establish an effective therapy that promotes the complete and effective regeneration of the injured peripheral nerve and consequent functional recovery. Several therapeutic alternatives have been developed and explored to achieve an ideal peripheral nerve regeneration after lesion. These new approaches include the use of cell therapies based on mesenchymal stem cells (MSCs) and biomaterial [2]. Previously, our research group demonstrated the benefits of using MSCs from the Olfactory Mucosa (OM-MSCs) and their secretome in combination with chitosan-based neural guide conduits (NGCs).

The sheep has recently been considered an adequate animal model for PNI studies. Besides being a species with great availability, easy to maintain and to manipulate, the sheep also presents peripheral nerves with anatomical and physiological characteristics identical to those of humans. Recent studies have made it possible to establish and validate different surgical injury protocols, therapeutic approaches, and functional assessment methods in this species, allowing the sheep to be used with greater confidence as a highly complex animal model. In this work, the use of the therapeutic combination between OM-MSCs secretome and chitosan-based NGCs was tested in promoting the regeneration of ovine peroneal common nerves.

Methods
12 animals received neurotmesis lesions, with an equal number of animals being treated with EtE sutures, NGCs and an NGC-OM-MSCs secretome combination. Over 24 weeks the animals were functionally evaluated regarding their mental state, posture, walking ability, sensitivity, and proprioception. At 1, 3 and 6 months the animals were also subjected to nerve and muscle ultrasound assessment, electromyography, and kinematic evaluation. After six months, the animals were euthanized and the healthy, intervened nerves and their respective cranial tibial muscles, as effector muscles, were harvested to be subjected to stereological and histomorphometric evaluation, respectively.

Results
The results obtained reveal significant improvements in the functional indicators evaluated throughout the study period, with both the use of NGCs and their combination with OM-MSCs secretome promoting a recovery in the evaluated parameters. Likewise, in *post-mortem* evaluations it was possible to identify signs of nerve regeneration and reversal of muscle atrophy in both groups.

Conclusions
In this trial, it was possible to test, in the ovine model previously developed by the research group, the pro-regenerative capacity of the secretome of OM-MSCs in combination with NGCs in promoting the regeneration of the transected common peroneal nerve through an *ante-mortem* functional evaluation and a *post-mortem* histological evaluation. In all assessments, both therapeutic approaches were able to promote an effective functional and structural recovery in the treated animals. New trials with a larger number of animals should be considered in the future to validate the results obtained here. Likewise, the use of a secretome subjected to preconditioning may modify the pro-regenerative properties of this therapeutic approach and should be tested in new future trials.

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Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_815 - Designing ECM-mimetic matrices to promote motor neuron maturation in 3D printed spinal cord models

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Introduction/Objectives
The extracellular matrix (ECM) is a pivotal yet often overlooked component of the central nervous system (CNS) microenvironment, playing a crucial role in neuronal maturation, signaling, plasticity, and aging. Current human in vitro systems face significant limitations in replicating neuronal maturation, which hinders their effectiveness for studying CNS injury, disease, and regenerative therapies. To address this challenge, various strategies have been developed to emulate the adult CNS microenvironment in vitro, including the use of generic ECM proteins (e.g., laminin and fibronectin), three-dimensional matrices like Matrigel, and astroglial feeder layers. Despite these advancements, a deeper understanding of the ECM-mediated spatiotemporal-specific signals regulating neuronal maturation and regeneration in the CNS remains essential. This study proposed a bottom-up approach to design rationally tailored ECM-mimetic materials by identifying developmentally relevant cues from spinal cord ECM to enhance the maturation of iPSC-derived motor neurons in both 2D and 3D printed systems.

Methods
Spinal cords from adult and postnatal mice were harvested and decellularized using a combination of ionic and non-ionic detergents, followed by mechanical agitation. To investigate the effects of aging on the decellularized ECM (dECM), coverslips were coated with either neonatal or adult dECM, and iPSC-derived motor neurons were seeded onto these surfaces. Differences in neuronal responses were analyzed using morphometric techniques and electrophysiological assays.

For further investigation, a proteomic analysis of ECM-enriched fractions from intact spinal cords identified three key candidates: Tenascin-R, Versican, and HAPLN1, that are a part of Perineuronal Nets in CNS. Recombinant forms of these proteins were selected as ECM candidates further studies. In 2D validation experiments, the three proteins were added with media to treat iPSC-derived motor neurons.

Next, for 3D validation, human-scale spinal cord scaffolds were designed and fabricated using volumetric 3D bioprinting techniques. The ECM-mimetic hydrogel for these scaffolds was formulated by combining the three PNN proteins with 1% hyaluronic acid (HA), forming a biologically relevant matrix for motor neuron maturation and functional analysis.

Results
Our findings revealed notable differences in the functional and regenerative properties of ECM derived from neonatal versus adult mice. Subsequent proteomic analysis of the spinal cord matrisome at these developmental stages highlighted three candidate molecules associated with perineuronal nets in the CNS ECM. Treatment with these proteins significantly promoted the maturation of human iPSC-derived motor neurons in 2D cultures. Validation in 3D systems using bioprinted spinal cord scaffolds further confirmed that the ECM-mimetic hydrogel enhanced the functional maturation of motor neurons, demonstrating its potential as a platform for CNS modeling and therapeutic development.

Conclusions
These findings indicate that the ECM-mimetic hydrogels developed in this study not only enhance synaptic plasticity but also effectively promote functional neuronal maturation. Additionally, this research highlights the potential of Matrisomal analysis as a valuable tool for uncovering a reservoir of therapeutic candidates for neurological applications.

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1074 - Microphysiological platform for modeling and treating traumatic central nervous system injuries

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Introduction/Objectives
Injuries to the central nervous system (CNS), including the brain and spinal cord, present significant challenges due to their complex and trauma severity-dependent cellular and molecular mechanisms. Traditional animal models often fail to replicate human physiological responses, limiting their relevance for clinical translation. Moreover, there is a critical need for advanced humanized in vitro platforms that can effectively mimic the dynamic processes underlying traumatic CNS injuries.

Methods
In this study, we developed a novel Traumatic Brain Injury (TBI) model using a microphysiological device fabricated from PDMS on a glass substrate. The device features two side channels for media flow and a central chamber equipped with an inlet to accommodate an impactor to create controlled contusion lesions. This innovative platform replicates heterogeneous co-cultures to mimic the complexity of brain tissue. Controlled trauma was induced using the impactor, and neuronal repair and regeneration mechanisms were evaluated. We then assessed the damage and investigated potential neuronal repair and regeneration mechanisms within the device by using polymeric nanoparticles (NPs) composed of poly(lactic-co-glycolic acid) functionalized with two different peptides: pVEC for enhancing transport across the spinal cord barrier and NCAM for promoting neuronal NPs internalization. The surface of the NPs was functionalized with polyethylene glycol to reduce rapid clearance. NPs were loaded with Riluzole, a sodium glutamate antagonist with favorable neuroprotective effects in preclinical models of traumatic spinal cord injury (SCI) and early-phase clinical trials.

Results
The study of microglia revealed their significant contribution to glial scar formation, where activation led to increased cytokine release, promoting thicker glial scars, neuronal retraction, and cell death. The therapeutic potential of riluzole-loaded nanoparticles (NPs) was evaluated within the microphisiological device, demonstrating superior outcomes compared to all other conditions, including free riluzole treatment alone. While riluzole alone showed some efficacy, it underperformed compared to NPs loaded with riluzole, which enhanced cell survival, neurite growth, and glial scar remodeling. In vivo administration of the riluzole-loaded nanoparticles in a mouse model of spinal cord compression showed significantly improved

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motor recovery, underscoring the superior efficacy of controlled drug release over free drug administration.

Conclusions
This new microphysiological platform provides a robust and reproducible method to investigate therapeutic treatments for CNS injury by providing a reliable and reproducible method to generate and analyze controlled brain injuries. This model is a valuable tool to advance research on TBI and improve the development of targeted therapies. This effective translation validates the potential of the microphysiological model as a critical bridge between basic research and clinical applications, accelerating the development of targeted therapies and improving their translational relevance.

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1158 - Highly flexible 3D printed gelatin-Pluronic scaffolds colonized with primary Schwann promote directional axonal growth for spinal cord regeneration.[2]

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Introduction/Objectives
Spinal cord injuries (SCI), primarily caused by trauma and accidents, affect approximately 0.5 million people worldwide each year, leading to total or partial loss of motor and sensory functions. This is largely attributed to the limited and slow regenerative capacity of the central nervous system (CNS), compared to the peripheral nervous system (PNS), which exhibits more efficient regeneration, mainly due to the presence of Schwann cells (SCs), which play a crucial role in facilitating axonal growth. The combination of biomimetic materials with neural or glial cells is particularly promising for reconnecting injured axons to their original targets. Our objective is to develop a 3D-printed biomimetic scaffold with high flexibility and mechanical properties matching those of the spinal cord, which supports primary Schwann cell (pSC) adhesion and alignment to promote directional axonal growth.

Methods
Our strategy involves the fabrication of tubular multichannel nerve guides (NGs) presenting an anisotropic architecture and topography using high-resolution stereolithography. To this aim, we combined different ratios of photosensitive gelatin methacryloyl (GelMA) and dimethacrylated pluronic (PluDMA), synthetized via methacrylation chemistry, to 3D-print NGs. The physicochemical and mechanical properties of the scaffolds were characterized, focusing on compression and suturing assays. Then pSCs extracted from rats sciatic nerves were seeded within the NGs channels, and their adhesion, proliferation and alignment along the channel direction were evaluated. Finally, Dorsal root ganglion (DRGs), also extracted form rats, were seeded on the pSCs-colonized NGs, and the axonal growth was assessed.

Results
First, GelMA and PluDMA were successfully synthesized, and combined with various ratios and polymer concentrations to print multichannel NGs with different channel diameters fitting design dimensions. The resulting scaffolds exhibited tunable mechanical properties in particular linked to Pluronic micellar structure that brings flexibility while maintaining a low Young modulus comparable to that of the Spinal cord, favorable for neural cells adhesion and proliferation. Mechanical characterization revealed that GelMA:PluDMA hybrid hydrogels demonstrated enhanced flexibility and improved resistance to stress compared to brittle only GelMA scaffolds and are easier to handle. Electron microscopy observations highlighted an internal microtopography aligned with the channel direction, a feature directly associated with the printing process. pSCs were efficiently and homogeneously seeded within the channels. Cellular analysis demonstrated efficient cells adhesion, coverage of the scaffolds channels and a high viability. Most importantly, pSCs align towards the channel direction independently of the channel diameter or cell density due to the microtopography. Furthermore, axonal growth within the channels was observed, with neurites sprouting from the DRGs.

Conclusions
To the best of our knowledge gelatin-Pluronic combination has not been reported to form scaffolds exhibiting mechanical properties matching those of the spinal cord. These NGs which support pSCs colonization and alignment while promoting directional axonal growth, hold significant promise for spinal cord regeneration.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1528 - 3D bioprinting of a blood brain barrier harnessing a novel microfluidic printing head

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Introduction/Objectives
The blood-brain barrier (BBB) is a critical obstacle in central nervous system (CNS) drug delivery due to its highly selective permeability. Current 3D *in vitro* models of BBB are based on microfluidic platforms[1]. These systems, albeit fundamental for research advancements, often fail to replicate the physiological characteristics of the BBB. 3D bioprinting has not been used yet to fabricate a human BBB[2]. This project aims to develop a novel 3D bioprinting strategy to fabricate a BBB model that addresses the limitations of current approaches and provides a robust platform for CNS drug screening and pathophysiological studies.

Methods
A one-step 3D bioprinting strategy is implemented using a custom-designed microfluidic printing head. The latter is developed as a monolithic system to create and extrude biocompatible fibers with exceptional control over resolution and deposition. The design integrates a mixing unit and two sequential flow-focusing regions to produce spatially patterned fibers with a stiff endothelial cell-laden shell surrounding a softer neural ink core. The endothelial ink incorporates a bioactive scaffold of decellularized extracellular matrix derived from the human placental chorion plate and human umbilical vascular endothelial cells. The neural ink includes iPSC-derived cortical NPCs in a biomaterial that ensures long-term culturing, and grants similar morphology compared to 2D monolayer cultures. The bioprinted structures were cultured in a customized bioreactor to perfuse media through the channel, mimicking physiological shear stress.

Results
Various 3D structures were successfully bioprinted with increasing numbers of layers and varying infill percentages, showcasing the robustness and precision of the biofabrication approach. By fine-tuning the bioink flow rates, distinct filament patterns were achieved, including core-shell and sandwich-like configurations. The latter pattern was prioritized for further investigation, given its potential to enhance neural connectivity across the 3D construct. Perfusion of culture media in the vessel induced endothelial cell elongation along the flow direction, a hallmark of physiological relevance. Neural precursors embedded in the softer core maintained high post-printing viability.

Conclusions
This novel 3D bioprinting approach paves the way for the realization of a physiologically relevant human BBB model that better

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replicates structural, mechanical, and cellular features of the native barrier compared to current on-chip systems. The model offers significant potential for CNS drug screening and studying BBB pathophysiology, overcoming key limitations of existing in vitro platforms.

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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_271 - Hybrid 3D-bioprinting towards an advanced bone healing model

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Objectives
Bone grafts are increasingly in demand due to a more active lifestyle, longer life expectancy, and rising osteoporosis cases. Although autologous bone grafts are currently considered the medical gold standard, their availability is limited and there is significant potential for donor site morbidity [1]. 3D bioprinting offers a promising concept to overcome these limitations through the spatially resolved assembly of a physiologically relevant bone construct. The aim of this work is to develop a novel hybrid bioprinting process that aims to print stabilizing bone cement (calcium phosphate cement, CPC), vascularization-promoting human umbilical vein endothelial cells (HUVECs) and hydrogel-based bioinks containing bone-promoting mesenchymal stem cells (MSCs). The complex approach includes a novel drop-on-demand (DoD)-based vascularization strategy established in our lab [2].

Methods
The design of the bone implant followed the physiological requirements of native bone tissue, where multiple printing technologies allowed to address the mechanical and biological properties of different bioinks. Two syringe-extrusion and one DoD-printhead were used simultaneously on a prototype hybrid bioprinter platform from BioFluidix GmbH (Freiburg, Germany) [3]. The DoD printhead (PipeJet® from BioFluidix) was specifically characterized and optimized to print high-density cell suspensions of up to 10^7 cells/ml. This allowed to introduce a novel concept of vascularization to the implant. Syringe-extrusion printing was used for cell-laden, bone-promoting hydrogels (specifically an osteo-hydrogel of fibrin, gelatin, hyaluronic acid, and glycerol) and stabilizing CPC.

Results
The final implant construct design consists of a four-layered structure with a circular, meandering CPC pattern and layers of cell-laden osteo-hydrogel and HUVEC bioink. The design and printer performance were evaluated in the individual layers before fabrication of the complete construct. MicroCT measurements of the printed construct showed successful achievement of a CPC structure with hydrogel-filled cavities. The modulus of elasticity of the bioprinted constructs was shown to be in the range of human bone (8 MPa ± 1.5 MPa). Live/dead viability tests confirmed cell viabilities above 75%, indicating that the bioprinting process did not harm the cells in individual steps or the overall printing process.

Conclusions
A complex bone implant construct featuring two different cell types, a specific osteo-hydrogel, and a stability-supporting physiological bone cement was successfully developed and realized by different bioprinting technologies in one process. The in vivo evaluation of such bone implant constructs is planned in an advanced orthotopic bone healing model of the rat.

Acknowledgments
This work is dedicated to the memory of Günter Finkenzeller, whose guidance, support, and inspiration were invaluable. Funding by the Deutsche Forschungsgemeinschaft (FI790/10-2, KO3910/1-2).

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Topic: Biomechanics & Mechanotransduction
Subtopic: Biomechanics
Type: Oral presentation

TERMIS25_530 - Auxetic Metamaterials for Skeletal Muscle Regeneration: Effects of Negative Poisson's Ratio on Muscle Maturation In Vitro

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Skeletal muscles enable voluntary movement, support posture and maintain overall body strength and mobility. Muscles are highly dynamic tissues, undergoing stretching that can in some cases reach up to 60% of their resting length. However, muscle function can be impaired by injuries and degenerative diseases. To induce effective muscle regeneration, scaffolds capable of matching its unique mechanical properties, while supporting cell growth and differentiation are needed.

Mechanical metamaterials are rationally designed structures engineered to exhibit extraordinary properties, which occur from the repetition of a single design block called the unit cell. Auxetic metamaterials are a class of metamaterials that exhibit negative Poisson's ratio (PR) (*i.e.,* they expand laterally when longitudinally stretched) and offer improved shear and indentation resistance – key properties for muscle scaffolds. This study investigates how auxetic metamaterial scaffolds influence myoblast maturation *in vitro,* presenting a novel approach to muscle regeneration.

Nine auxetic geometries were modeled mathematically to determine optimal geometric parameters influencing the auxeticity degree. Key factors including porosity, pore size, and relative density were theoretically tuned to support cell seeding. The optimized geometries were then translated into auxetic scaffolds using Grasshopper and a custom slicing algorithm. COMSOL simulations were performed to predict the mechanical response of the auxetic lattices under tensile loading. Scaffolds were printed with a melt-extrusion printer using a PCL/PLLA copolymer, and their morphological parameters were assessed by SEM imaging. Force-displacement curves were extracted per geometry using tensile tests, while the lateral displacement of the structures was calculated using a custom computer-vision algorithm. Mouse myoblasts (C2C12) were seeded in selected conditions and their viability and metabolic activity were quantified using the DNA and Presto Blue assays. The scaffolds were stained for myogenic differentiation markers (myogenin, myosin). Maturation was assessed by fusion index estimation (percentage of multinucleated myosin-positive cells). Cell alignment was also quantified using orientation algorithms.

Theoretical and experimental geometrical parameters aligned closely, confirming that the mathematically predicted auxetic behavior was preserved in the printed scaffolds. Stress-strain curves revealed a geometry dependence of the elastic moduli, while PR analysis showed that eight out of the nine geometries exhibited auxetic behavior for strains below 10%, with some maintaining this response for up to 100% deformation. After the mechanical screening of all the conditions, three geometries -tetrachiral, Lozenge and arrowhead- were selected for biological evaluation. These designs exhibited a similar elastic modulus while having different PR, allowing us to decouple the effect of stiffness from that of the auxetic behavior on the cells. Enhanced myoblast attachment, proliferation,

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differentiation and maturation was observed in the constructs with the most negative PRs.

By decoupling stiffness from auxeticity, we show that auxetic metamaterials provide a favorable biomechanical environment that could enhance muscle regeneration. This work paves the way for future research on the integration of auxetic metamaterials into functional muscle repair applications.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_617 - in situ bioprinting of craniofacial bone: preclinical study in a live rabbit model

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Introduction/Objectives
In situ bioprinting of bone represents a transformative approach in the field of regenerative medicine to directly repair and regenerate bone tissues at the injury site. Unlike traditional bone grafting methods, in situ bioprinting offers the advantage of real-time customization, enabling tailored scaffolds that conform to the unique geometry of each defect and integrate seamlessly with surrounding native tissues.

Recent advancements in handheld bioprinting devices have showcased an innovative approach for in situ applications where precision is less critical than adaptability. While handheld bioprinters can accommodate irregular defect shapes, their output may vary due to manual control, making them less suitable for situations where exact spatial arrangement and complex structures are essential.

The use of in situ bioprinting with a traditional 3D Bioprinter device is particularly advantageous in terms of accuracy and practicality, in complex or irregularly shaped bone defects- which are difficult to treat using conventional methods. Furthermore, in situ bioprinting minimizes the risk of contamination and reduces surgical time, making it a promising approach for point-of-care applications in orthopedic surgery.

Methods
In this study, critical-sized bone defects were created on the parietal bones of rabbits. An autologous adipose-derived stem cell-laden alginate/hydroxyapatite bioink was applied using a 3D bioprinter (Envisiontech Bioplotter) to the defects in the animals under anesthesia. Control groups were applied as non-cell bioink and sham. Post-operative evaluations included micro-CT scanning and histopathological analysis to assess bone healing and bone-material integration.

Results
The results demonstrated successful bone regeneration with the in situ bioprinting approach, as compared to the sham group and the group using bioink-only. Quantitative analyses in micro CT revealed that the cellular group had the highest bone volume and percent bone volume, followed by the acellular group, and the sham. The bone surface/volume ratio and bone surface density were higher in both the cellular and acellular groups compared to the sham, indicating better bone formation and coverage.

In defects filled with acellular material, a thin capsule structure was observed around the material, primarily consisting of fibrocytes. Additionally, periosteal proliferations were seen extending into and around the material, indicating a response to the implanted scaffold. In contrast, defects filled with cellular material exhibited a thicker capsule structure, primarily composed of fibroblasts and collagen fibers. This group showed numerous segmented and/or pyknotic neutrophils on the capsule and severe inflammatory cell infiltration immediately outside the capsule, consisting of lymphocytes, plasma cells, and a few macrophages. In the sham, primarily fibrocytes and collagen fibers were observed, suggesting an attempt at repair rather than proper bone regeneration.

Conclusions
The novelty of this research lied in the direct application of bioprinting onto live animals under anesthesia, which enhances the clinical relevance of the findings and paves the way for future clinical applications.

Acknowledgements
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Topic: Bioprinting & Biofabrication
Subtopic: 4D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_750 - 4D biofabrication of magnetically augmented callus assembloid implants enables rapid endochondral ossification via activation of mechanosensitive pathways

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Introduction/Objectives
Magnetically responsive living systems have advanced tissue engineering and regenerative medicine in the context of bone regenaration by enabling remote control, actuation, stimulation and by enebling cellular functionalities through magnetic nanoparticles (MNPs) and magnetic fields. MNPs have been incorporated into hydrogels, polymer fibers, and spheroids to create magnetically guided biofabrication strategies, facilitating the assembly of living materials with inherent magnetic properties. These setups drive morphogenetic events and shape transformation in 4D, while influencing biological processes such as differentiation. However, long-term effects of magnetic stimulation on tissue functionality and *in vivo* performance of implants remain underexplored.

Methods
Human periosteum-derived cells (hPDCs) were expanded in 2D for 7 days before being seeded on AggreWell™400 to form 3D callus microtissues in a chondrogenic medium (C8). After 7 days, microtissues were incubated with iron oxide-based MNPs a day prior to biofabrication. A neodymium magnetic plate (24-WELL BIO ASSEMBLER™) generated a strong magnetic field to assemble callus assembloids. Samples were collected at various stages of in vitro maturation under magnetic stimulation, with non-magnetic controls for comparison. Ectopic implantation at two maturation stages was analyzed using histology, confocal microscopy, SEM, and μCT. Magnetic field characterization and SQUID magnetometer analysis

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quantified IONPs. Combined data, including videos of the biofabrication process, informed a computational model simulating magnetically driven assembly of our set up. RNA sequencing was used to identify key gene expression transitions between stimulated and control samples.

Results
Cell viability and toxicity were unaffected by MNPs throughout biofabrication and maturation. Histological analysis revealed matured hypertrophic chondrocytes and ECM components (SafO+) in samples undergoing chondrogenic differentiation under magnetic stimulation. SEM-EDX and SQUID magnetometer analysis localized and quantified IONPs within ECM compartments. Ectopic implantation showed early bone formation in magnetic-stimulated assembloids. Both stimulated and non-stimulated groups formed bone compartments in the late time point, with early non-stimulated explants exhibiting poor bone-forming capacity. The computational model efficiently recapitulated the magnetic assembly experiments. RNA seq analysis showcased in the magnetic stimulated group, upregulation of mechanosresponsive pathways associated with endochondral ossification.

Conclusions
Our study demonstrated that magnetically augmented cartilaginous microtissues enable precise biofabrication of functional callus assembloid implants. Magnetic stimulation enhanced chondrogenic differentiation at the gene and ECM levels, improving bone-forming capacity upon in vivo implantation. Stimulated assembloids formed complete ossicles earlier than controls, highlighting their role in guiding bone formation. Transcriptome analysis revealed mechanoresponsive pathways driving endochondral ossification, highlighting potential therapeutic targets. This approach offers a controlled and less invasive delivery method by leveraging the inherent magnetic properties of these responsive living systems.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1199 - Development of collagen-based scaffolds for bone tissue engineering incorporating antimicrobial KR-12 peptide derivatives

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Introduction/Objectives
Additive manufacturing enables the creation of complex, patient-specific structures that closely mimic bone morphology that could be used for the for the treatment of critical size bone defects. Collagen is widely used in bone tissue engineering, but its use is limited by low mechanical strength. The mechanical properties of collagen can be enhanced by polymer chemical modifications and crosslinking. A significant challenge in orthopedics is bacterial infection; thus, implants with innate antimicrobial qualities are advantageous[1]. In this context, antimicrobial peptides (AMPs) present a promising solution due to their biocompatibility, broad-spectrum antibacterial activity and reduced probability of inducing bacterial resistance[2]. Optimizing the viscosity and flow properties of collagen modified with AMPs is crucial for 3D printing through direct ink writing. This study aims to incorporate derivatives of the AMP "KR-12" into collagen scaffolds intended for bone regeneration.

Methods
KR-12 derivatives were covalently incorporated into collagen type I using two chemical approaches: EDC/NHS coupling and thiol-ene maleimide chemistry. The KR-12 derivatives, modified to enhance their activity, were labeled with biotin to quantify the incorporated peptide using a HABA/Avidin assay. The modified collagen with KR-12, intended for use as biomaterial ink, was optimized for printing by adjusting the concentration and printer settings. An open-source bioprinter[3] was used to manufacture collagen scaffolds that were biologically characterized for cytocompatibility with human osteoblasts (hOB) indicated by cytosolic lactate dehydrogenase (LDH), osteoblast activity, measured by alkaline phosphatase (ALP), and subjected to antimicrobial assays.

Results
KR-12 derivatives were successfully incorporated into collagen using two different chemical approaches, achieving the incorporation of 60-80 mg KR-12 peptide /g dry material. The developed materials were optimized for their use as biomaterial ink, making them suitable for direct ink writing. Additionally, the growth of human osteoblasts (hOB) cultured on collagen-KR12 scaffolds was evident from confocal imaging and high levels of LDH production.

Conclusions
Collagen-based materials incorporating the KR-12 antimicrobial peptide were produced, demonstrating good printability and suitability for direct ink writing. These materials were used to create antimicrobial scaffolds for use in bone tissue engineering and to potentially combat bacterial infection. This development is promising for the future, opening the possibility of incorporating more potent peptides and creating more complex 3D-printed structures.

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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1254 - Textile-Inspired Biofabrication of Cell-Laden Microfibers for Scalable Artificial Skeletal Muscle Engineering

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Introduction/Objectives
Recent progress in biofabrication enables the creation of scaffolds with defined shapes and microstructures. Notably, cell-laden hydrogels allow for precise positioning of different cell types within engineered tissue. Despite achievements in cell survival and functional restoration, the rapid production of large, mechanically stable constructs remains a significant challenge.

This study introduces an innovative approach to creating multifunctional microfibers processed into fiber-like 3D structures several centimeters long using simple textile technology. To validate this concept, we applied this method to produce artificial skeletal muscle tissue from primary human skeletal muscle (HSKMC) and endothelial cells (HUAEC). A comparative study evaluated three bioinks based on collagen, alginate, and/or gelatin for their effectiveness in supporting skeletal muscle growth.

Methods
Cell-laden filaments, composed of three distinct cell inks, were printed under sterile conditions. The filaments contained 1.5×10^6 cells/mL and were cultivated in cell-type-specific media. HUAEC were cultured for 14 days, while HSKMC were cultured for 38 days, both until achieving complete confluence on the filament surface. Following this, the filaments were manually woven and co-cultured for a minimum of 7 days. Proliferation and viability were assessed using Live-Dead staining and Phalloidin-DAPI staining.

Results
The 3D-printed, cell-laden filaments were pre-cultured to enhance mechanical strength and increase cell density. Skeletal muscle cells exhibited slow proliferation rates in alginate-based bioinks. All cell-laden microfilaments were resilient enough to be manually woven into a stable lattice. The resulting constructs were cultivated over several days. Immunofluorescent analysis showed high cell survival, parallel alignment of skeletal muscle cells, and rapid endothelial cell

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migration into a tubular structure. Cell morphology was observed at filament intersections. Furthermore, myogenic differentiation was investigated by examining the expression of myogenin and MyoD.

Conclusions
Our approach combines straightforward 3D printing and weaving techniques, offering an efficient biofabrication method for in vitro reconstruction of fibrous artificial skeletal muscle tissue. The large size of the resulting constructs suggests potential applications in treating substantial skeletal muscle loss.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1304 - Enabling high fidelity 3D printing of bone mimetic nanocomposite hydrogels by enhancing rheological properties using polyacrylic acid

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Introduction/Objectives
Critical-size bone defects resulting from traumatic injury, degenerative disease or surgical removal of a tumour, need clinical intervention for complete healing. While conventional treatment options like autografts, allografts and metallic bone fixation devices pose several risks, bone tissue engineering via 3D extrusion-based printing is widely discussed as an alternative approach. However, many extrudable materials designed for bone regeneration do not yield high resolutions due to inadequate rheological properties of the inks. Our objective was to address these limitations by introducing a novel composition capable of chemically, mechanically and structurally mimicking bone tissue.

Methods
For this purpose, we developed a novel bone-mimetic ink based on methacrylated gelatin and nanohydroxyapatite (nHAp). Hydroxyapatite constitutes the inorganic component of the natural bone matrix, while gelatine is derived from the hydrolysis of collagen which makes up the majority of the organic part of the bone matrix. The functionalisation of gelatin with methacrylate groups enables crosslinking of gelatin backbones using UV light in the presence of the photoinitiator irgacure, ensuring rapid and uniform covalent bonding. Another crucial component is the rheological modifier carbomer. The polyacrylic acid enhances ink rheology by absorbing free Ca^{2+} ions released by nHAp. Using mechanical, rheological and morphological characterisation techniques, we analyzed the influence of the main components on printability, swelling, moduli and stiffness of the nanocomposite hydrogel (NCH). Additionally, cytocompatibility and osteogenic differentiation on the printed scaffolds was investigated in *in vitro* experiments using mesenchymal stem/stromal cells (MSC). Further, dispersion of nHAp particles as well as the morphology of MSCs growing on the scaffolds was investigated using EDX mapping and SEM. Ongoing *in vivo* experiments are being carried out to evaluate the induction of local and systemic effects of the printed constructs in mice.

Results
The NCH allowed 3D printing with high resolution and shape fidelity, resulting in scaffolds with fully interconnected macropores and well-dispersed nHAp particles. After mechanical deformation by bending or squeezing, the printed constructs regained the original without losing structural integrity. The uniformity as well as the printability factor of 3D printed filaments showed values close to 1 which underlines the promising rheological characteristics while μCT measurements revealed a distribution of pore sizes ranging from < 5 μm up to 20 μm in fully swollen filaments. Live/Dead staining experiments and metabolical tests over 72 h additionally confirmed a high cytocompatiblity of the cured scaffolds and osteogenic differentiation of MSC *in vitro* was shown by ALP stainings. SEM imaging confirmed a dense colonization of the scaffolds with visible cell-material interactions by cellular protrusions.

Conclusions
Overall, we present the development and characterization of a novel bone-mimetic ink that allows osteogenic differentiation and has a cost-efficient formulation. Its 3D printability with high shape fidelity allows for customization and the resulting flexible structure after printing enables foldable designs e.g. for minimally invasive treatments. Thus, the material has promising characteristics to improve future treatment options for critical-size bone defects.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_1437 - Hybrid supramolecular-covalent gelatin bioresins as a platform for high-fidelity bioprinting and bone marrow vascular niche modeling

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Introduction/Objectives
Bioprinting has become a powerful tool in biomedical research, enabling the creation of 3D models with intricate architectures and spatially defined cell niches. However, achieving high shape fidelity bioprinting of cell-laden biomaterials with low mechanical properties (~500-1500 Pa), which better support cellular functions, remains a significant challenge. Although mechanically stable, covalently crosslinked hydrogels, limit cell migration and organization, potentially hindering biological processes like capillary network formation. Conversely, hydrogels based on supramolecular reversible bonds are more permissive to cellular activity but suffer from limited shape stability. This study aims at introducing microvascular networks in a gelatin-based hybrid biomaterial that combines the advantages of covalent and supramolecular bonds. As a showcase application, the vascularized hybrid hydrogels were employed as bone marrow (BM) vascular niche biomimetics, to study hematopoietic stem cell (HSC) engraftment and differentiation.

Methods
Gelatin-methacryloyl (covalent GelMA), with varying degrees of methacrylation (DoM = 60-100%), was functionalized with adamantane to produce hybrid GelMA. Acrylated-β-cyclodextrin was incorporated in the pre-polymer solution, with lithiumphenyl(2,4,6-trimethylbenzoyl)-phosphinate as photoinitiator. Mechanical properties and rheological behaviour of covalent and hybrid hydrogels were characterized. Shape fidelity of different formulations was evaluated by fabricating complex architectures using a volumetric 3D printer (Tomolite Readily3D). Mesenchymal stem cells (MSCs) were used to assess cell migration and cell-biomaterial interactions. Additionally, MSCs were combined with endothelial colony-forming cells (ECFCs) in a 3:1 ratio to generate vascular spheroids, which were bioprinted within the hybrid GelMA to mimic BM vascular niche. Fluorescently labelled $CD34^+$ HSCs migration within the niche biomimetic was assessed via microscopy, and HSCs differentiation was monitored through flow cytometry.

Results
Hybrid hydrogels presenting supramolecular host-guest interactions and covalent bonds were formed upon exposure to visible light through methacryloyl chain-growth polymerization. Hybrid hydrogels possessed a significantly higher compressive modulus than covalent hydrogels with equivalent DoM, leading to improved shape fidelity in printed 3D structures. An increase in loss modulus and tan δ was also observed in the hybrid groups, which correlated with an increased MSCs migration. MSCs embedded within the hybrid hydrogels also displayed reduced circularity and increased focal adhesion formation. In co-culture experiments, MSC-ECFC spheroids embedded within hybrid GelMA formed interconnected vascular networks. HSCs showed significantly higher engraftment within the hydrogels containing spheroids, compared to cell-free controls. Notably, after three days, HSCs cultured within the

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vascular niche biomimetic exhibited an increase in common myeloid progenitor cells, while those in cell-free hybrid hydrogels maintained a higher percentage of multipotent progenitors.

Conclusions
The integration of covalent and supramolecular bonds preserved shape fidelity in soft gels while supporting the development of a microcapillary network, thereby enabling the engineering of a 3D platform to study interactions between HSCs and vascular BM niche.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_1450 - Generative design, advanced 3d printing, and computational modeling for bone-mimetic hydrogel scaffolds: design and assessment

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Introduction/Objectives
The growing incidence of bone fractures and osteoporosis necessitates innovative bone regeneration strategies. This study develops bio-inspired scaffolds using a generative design informed by synchrotron-based analysis of healthy and osteoporotic trabecular bone, optimizing fabrication, mechanics, and biocompatibility for bioreactor applications.

Methods
Scaffold geometries were designed based on synchrotron high-resolution data using Voronoi tessellation to mimic trabecular bone porosity and architecture, providing simplified yet controllable structures. Xolography, an advanced volumetric 3D printing technique, was used to fabricate scaffolds from hydrogels comprising polyethylene glycol diacrylate (PEGDA) and dimethacrylate (PEGDMA), functionalized with the arginine-glycine-aspartate (RGD) peptide sequence to enhance cell adhesion. Xolography's unique capability to fabricate entire volumetric structures at sub-micrometric resolution in a single step offers unparalleled precision and speed, but also presents challenges in material optimization and process stability.

Results
A range of PEGDA and PEGDMA compositions was tested for printability, mechanical properties, and cytocompatibility, with 30% PEGDA–10% PEGDMA identified as optimal.

Scaffold permeability and shear stress were modeled using computational fluid dynamics (CFD) simulations to predict performance in a perfusion bioreactor. Scaffold porosity and permeability were compared between healthy and osteoporotic designs. Xolography enabled the fabrication of high-resolution scaffolds with porosity tailored to match trabecular bone conditions. Mechanical tests revealed compressive moduli ranging from 1.2 to 3.4 MPa, compatible with native bone tissue. CFD simulations demonstrated permeability values between 3.54×10^{-8} and 19.0×10^{-8} m^2, consistent with natural bone. Healthy bone-inspired scaffolds exhibited lower permeability but higher shear stress distribution than osteoporotic scaffolds, which presented more osteogenic zones without achieving sufficient mineralization stimuli due to reduced density. Cell viability tests confirmed over 85% viability in RGD-functionalized 30% PEGDA–10% PEGDMA scaffolds, balancing resolution and biocompatibility.

Conclusions
This study establishes a robust workflow integrating bio-inspired design, advanced 3D printing, and computational modeling: Voronoi-based geometries and PEGDA-PEGDMA hydrogels demonstrate a balance between structural fidelity, mechanical strength, and biological performance. CFD simulations provide actionable insights for tuning scaffold permeability and shear stress in bioreactor applications, guiding further development of patient-specific scaffolds. These findings offer a significant step forward in addressing critical-size bone defects.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_1491 - Mimicking tumor microenvironment to model metastatic Colorectal Cancer (mCRC) patient-derived organoids dynamics

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Introduction/Objectives
Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide and the 5-year relative overall survival for metastatic patients remains poor. This study introduces a novel matrix based on gelatin methacrylyol (GelMA) for enhancing 3D models of patient-derived metastatic colorectal cancer (mCRC). Traditional 2D cell cultures inadequately replicate the complex tumor microenvironment, limiting the accuracy of preclinical drug testing and cancer research. The newly developed GelMA-based matrix aims to overcome these limitations by providing a more physiologically relevant scaffold that mimics the extracellular matrix of mCRC.

Methods
We worked with five patient-derived mCRC organoids from the XENTURION platform shared by prof. L. Trusolino. All the organoids derived from liver metastatic, heavily mutated and resistant CRC tumor and showed the same transcriptional profile and sensibility to drugs of the original tumor. The GelMA-based matrix was synthesized and optimized through in vitro 3D proliferation essays, for mechanical properties and biocompatibility, supporting realistic cell morphology, proliferation, and invasion behaviors. We tested two different types of GelMA-based matrix (porcine A and bovine B) and three different compositions for each one.

Results
In vitro assays demonstrated that the GelMA A-based matrix outperforms existing 3D culture systems in maintaining essential cellular functions and interactions. Two-week 3D cultures in the GelMA A-based matrix showed that patient-derived mCRC organoids proliferated and organized into typical tube-like structures, displaying good interactions and high vitality upon confocal analysis.

Conclusions
The next steps involve performing long-term 3D cultures in the GelMA-based matrix and subsequently confirming the tight similarity in the transcriptional profile and drug response of the patient-derived mCRC organoids with corresponding original tumors. Ultimately, our results lay the groundwork for presenting our GelMA A-based matrix as a viable alternative to conventional options like Matrigel and BME.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1542 - Bioprinted complex constructs enhance osteogenesis and angiogenesis in dynamic conditions

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Introduction/Objectives
Osteogenesis and angiogenesis are coupled in a spatiotemporal and inter-dependent manner during bone regeneration. We introduce a complex 3D bioprinted model for the biofabricaton of a holistic

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vascularized bone model, comprising (i) an outer zone from a photocrosslinkable hydrogel with conductive properties to enhance osteogenesis under dynamic conditions, and (ii) an inner zone including a vascular-like tubular structure from a biofunctional nanocomposite hydrogel to induce angiogenesis. The objective is to bioengineer a complex 3D bioprinted vascularized bone model and evaluate the synergistic interactions of the functional bioinks in dynamic cell cultures, hypothesizing that paracrine signals from osteogenic and angiogenic processes derived from the crosstalk between the two mesenchymal stem cell types of the combined constructs will accelerate bone healing.

Methods
The angiogenic matrix was prepared by dispersing gellan gum in a laponite suspension. Wharton's jelly mesenchymal stem cells (WJ-MSCs) were incorporated in the nanocomposite forming a bioink functionalized with platelet-rich plasma (PRP). The osteogenic matrix was prepared from a blend of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), polyvinyl alcohol, gelatin and polyethylene glycol diacrylate combined with bone marrow mesenchymal stem cells (BM-MSCs). Biofabrication of vascularized bone model was achieved through 3D bioprinting. For dynamic cultures, mechanical stimulation (1 Hz, 10% strain, 1 h) was applied daily for 28 days. Biocompatibility study included Live/Dead assay and evaluation of ECM formation. Angiogenic and osteogenic differentiation were examined through immunofluorescent staining, determination of VEGF/BMP-2 secretion by ELISA, gene expression via quantitative polymerase chain reaction (qPCR), ALP staining, calcium and collagen quantification. Subcutaneous implantation of the constructs was performed for two weeks.

Results
Bioinks maintained over 90% cell viability from day 1 to day 9, and were positive for all ECM markers. At day 28, qPCR analysis demonstrated that in dynamic conditions the complex model exhibited remarkable increases in TIE1 (11-fold), Ang1 (7-fold), VEGFA (7-fold), and VEGFR2 (5-fold) expression levels, indicating a heightened angiogenic potential. Also, a remarkable increase in key osteogenic markers, including BGLAP (10-fold), SPARC (11-fold), OPG (8-fold), and RUNX2 (7-fold), highlighting the enhanced osteogenic potential of the biofabricated constructs under dynamic culture conditions. For the same period ELISA quantification demonstrated that the complex model in dynamic conditions consistently yielding higher VEGF concentrations (313 pg/ml). Similarly, BMP-2 release in composite dynamic setups peaked at 28 ng highlighting pronounced impact of dynamic culturing on sustaining and enhancing BMP-2 release. Immunostaining at 2 and 4 weeks confirmed positive osteogenesis in biofabricated constructs from day 14, with dynamic conditions, notably with PEDOT:PSS, showing enhanced effects, suggesting mechanotransduction pathway activation. No adverse reaction or immune response was reported in vivo.

Conclusions
The data support the hypothesis that paracrine signals from angiogenic and osteogenic pathways, particularly under dynamic culture conditions, contribute to accelerated bone healing.

Topic: Submit to SYMPOSIUM
Subtopic: 4D tissue engineering: widening the control over cells microenvironment
Type: Oral presentation

TERMIS25_745 - Xolographic (bio)printing for spatial control of material properties in 3D hydrogels

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Introduction/Objectives
Stiffness differences are common in native tissues and play a key role in steering cell behavior *in vitro*.[1] Traditional biomaterials however lack the ability to provide spatially controlled stiffness in order to guide cell fate and tissue development. Recent advances in photopolymerization-based 3D printing technologies now enable manufacturing of tissue-scale scaffolds with anisotropic stiffness distribution in a single print. Processes like FLight bioprinting control energy exposure and thus influence photopolymerization to create aligned microfibers. They however do not yet allow stiffness control along three axes, which is essential for capturing the cues from instructive extracellular domains *in vivo*.[2] We herein suggest the use of the two intersecting light sources of a dual-color light-sheet volumetric printing process, xolography, to locally tune energy dose and achieve control over stiffness in both cell-free and cell-laden biomaterials along three dimensions.[3]

Methods
We employed xolography to create scaffolds based on polyethylene glycol diacrylate (PEGDA) and gelatin methacryloyl. We validated the influence of energy dose on degree of crosslinking with sol fraction and Fourier-transform infrared spectroscopy and assessed differences in stiffness with compression testing, following a design of experiments approach. We adjusted irradiation intensity by transitioning from a binary to a grayscaled projection, which, in combination with orthogonally applied irradiation in the ultraviolet (UV) spectrum, allowed to modulate energy dose locally. We conducted stress relaxation experiments to investigate viscoelastic material properties. Nanoindentation was employed to determine local stiffness in terms of Hertzian Young's moduli. We assessed cytocompatibility of scaffolds for aggregates of multipotent human mesenchymal stromal cells through metabolic activity assays and fluorescent imaging.

Results
We developed cytocompatible hydrogels for xolography. The addition of PEGDA improved the printing accuracy but reduced cytocompatibility. We manufactured scaffolds that contained viable cell aggregates which developed morphologies dependent on their location. We modulated stiffness of printed hydrogels from kilopascal to megapascal by manipulating UV energy dose between 2 and 8 mJ/mm^2. The variation of UV energy dose furthermore led to differences in relaxation half time. Grayscaled projections based on a pixel size of 4.6 μm allowed to incorporate regions of varying stiffness in a single part which were generated by doubling the projected visible light intensity from 120 to 240 mW/mm^2. We further created microscale stiffness gradients of 20 Pa/μm.

Conclusions
3D stiffness gradients were implemented *in situ* in centimeter-scale constructs employing a single hydrogel-based photoresin at printing rates above 300 mm^3/min. Achieved stiffness covered the range from soft tissue like brain to stiffer tissues like cartilage and thus demonstrated physiological relevance. We presented a novel biomaterials manufacturing method to create scaffolds from cytocompatible hydrogels with controlled heterogeneity in four dimensions (3D geometry and material properties) to potentially guide cell fate and tissue development in space, opening new perspectives for 4D tissue engineering.

[1] Huebsch et al. Nat Mater 9 (2010).
[2] Puiggalí et al. Adv Healthcare Mater 13 (2024).
[3] Regehly et al. Nature 588 (2020).

Topic: Bioprinting & Biofabrication
Subtopic: 4D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_864 - Enhancing mechanical properties of pediatric tissue-engineered heart valves: a 28-day dynamic culture study

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Introduction/Objectives
Each year, thousands of children born with a defective heart valve must undergo replacement surgery. Unfortunately, the substitutes

currently used cannot grow along with the child. Tissue-engineered heart valve substitutes, made from living cells, might solve this issue. However, current methods struggle to reproduce and maintain the complex three-dimensional anatomy of the valve throughout the entire tissue engineering process. Therefore, our objectives are: 1) to develop a rapid biofabrication method for personalized, cellularized heart valves that reproduces the valve anatomy and preserves it throughout the fabrication period; 2) to culture the valves dynamically for up to 28 days; 3) to evaluate the mechanical properties of the valves over time.

Methods
A pulmonary heart valve with custom dimensions for pediatric patients was modeled. Then, an innovative biofabrication method featuring a sutureless design was developed to produce cellularized fibrin-based heart valves. A total of 18 valves were manufactured. Three acellular (n=3, fibrin scaffold only) and cellularized valves (n=3, human adipose-derived stromal cells) were produced for each analysis time-point: on the day of fabrication (day 0) and after 14 and 28 days of dynamic culture in a specially designed bioreactor. The culture method allowed to avoid direct manipulation of the construct while providing controlled tissue contraction to preserve the valve geometry during maturation. Cell viability was assessed on day 0 to evaluate the impact of the technique. The mechanical properties of both acellular and cellularized valves were assessed via puncture testing after 14 and 28 days in the bioreactor. The valve thickness was measured using an optical micrometer at the same time points. Each analysis was performed on 3 samples per valve of the leaflets and root (pulmonary trunk).

Results
Each cellularized valve was engineered in just 20 minutes. The fabrication steps did not affect cell viability, which was above 90 % at day 0. After 28 days of culture in the bioreactor, the valve leaflets had a stiffness nearly 5 times greater (913 ± 155 mN/mm) than the valves cultured for 14 days (200 ± 65 mN/mm) and nearly 10 times greater than the acellular valves also cultured for 28 days (92 ± 19 mN/mm). The leaflets had an average thickness of 394 ± 19 μm after 28 days, similar to values reported for human leaflets. Each matured valve had an approximate diameter of 16 mm.

Conclusions
This innovative biofabrication method of a pulmonary heart valve substitute leverages the known benefits of fibrin for cell-mediated tissue production while mitigating the contraction associated with its use. The enhanced mechanical properties observed after 28 days of culture suggest that this approach represents a novel and promising solution to the challenges of engineering pediatric heart valves. Further maturation and durability assessments in a pulsed cardiac bioreactor are needed to confirm both functionality and potential for implantation.

Topic: Bioprinting & Biofabrication
Subtopic: 4D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1194 - Innovative in situ 4D bioprinting for regeneration of colorectal mucosa and submucosa

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Introduction/Objectives: Colorectal diseases are a cohort of pathologies that affect the mucosa and submucosa layers of the anus, rectum, and colon of more than 2 million individuals in the European Union. Among them, familiar adenomatous polyposis (FAP) and ulcerative colitis (UC) seriously compromise the patient's quality of life. These pathologies could benefit from removing the intestinal mucosa and submucosa. However, no strategies exist nowadays to replace them. Then, when their removal is necessary, the patients undergo a proctocolectomy (i.e., surgical removal of the rectum and all or part of the colon) with a subsequent ileal pouch. Although preserving the patient's continence, the procedure is burdened by significant complications. Thus, in this context of the EU funded project "TENTACLE", we envision a radically new strategy for the surgical treatment of UC and FAP, by developing an innovative in situ 4D bioprinting suite to fabricate a shape-morphing layered bioconstructs directly inside the patient rectum/colon, able to replicate the human colorectal mucosa and submucosa. These bioconstructs will promote the regeneration of colorectal mucosa and submucosa thanks to their composition and the delivery of specific active pharmaceutical ingredients, such as antibiotics and antifibrotics. Moreover, the mucosa layer will possess a shape-morphing capability, triggered by humidity, that will induce the creation of crypts on its surface, mimicking the colorectal ones.
Methods: TENTACLE in situ 4D bioprinting suite will comprise a first-of-its-kind colonoscopic bioprinter that will be inserted on commercial colonoscopes and will feature an extrusion-based bioprinting unit, a valvejet printhead, and a photocrosslinking apparatus. Multiple artificial intelligent empowered in silico tools will be developed to improve the performance of the bioprinting suite. More in detail, an algorithm to build the bioconstructs' geometry on the patient defect will be created starting from the patient medical image. Then, a second algorithm for path planning, namely the ideal distribution of ink, will be developed. Finally, an algorithm to real-time monitor the in situ bioprinting process via image analysis will be implemented. Regarding the inks, two novel bioink formulations containing patients' cells will be developed, based on photocrosslinkable gelatin and thiolated polysaccharides. Those bioinks will be enriched with engineered micro- and nano-carriers incorporating antibiotic and anti-fibrotic agents.
Results: The entire in situ 4D bioprinting procedure will be validated in vitro, ex vivo and in vivo, thus paving the way for translating the bioprinting suite toward the clinics.
Conclusions: Collectively, TENTACLE will introduce a minimally invasive alternative to proctocolectomy and is expected to have a high impact on the quality of life of patients affected by FAP and UC.

Topic: Submit to SYMPOSIUM
Subtopic: 4D tissue engineering: widening the control over cells microenvironment
Type: Oral presentation

TERMIS25_1513 - Effects of surface area on the cytotoxicity of 3D printed magnesium-, zinc-, and nitinol-based implants: predicting in vivo toxicity

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Introduction/Objectives
Although magnesium, zinc, and nitinol are biocompatible metals, the degradation of magnesium- and zinc-based implants has the potential to lead to toxicity. The goal of this study was to evaluate the cytotoxicity of magnesium and zinc in long-term degradation studies in vitro, including their combinations with each other and with nitinol, aiming to predict in vivo toxicity in a mouse subcutaneous model as a first step towards clinical translation of multimaterial implants.

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Methods
Prototypes were designed with a high surface area (squares of 6mm x 6mm or disks of 6mm diameter) or a low surface area (disks with diameter of 3-3.5mm), all with a thickness of 1mm. Samples from the magnesium alloy WE43 or a zinc alloy with 1 wt% magnesium (Zn1Mg) were additively manufactured using the laser powder bed fusion (LPBF) machine, Aconity MIDI+, and those from nitinol were manufactured using LPBF with the RenAM 500QFlex. Additional samples were manufactured by conventional machining. Some samples were further coated by plasma electrolytic oxidation (PEO). To study degradation in vitro, samples were incubated in cell culture medium for 25 days, with daily medium changes, followed by evaluation of ion release using inductively coupled plasma optical emission spectroscopy (ICP-OES), analysis of morphology by scanning electron microscopy (SEM), and cytotoxicity assessment through indirect tests with SaOS-2 cells. The volume of medium used in the extraction was varied, as was the incubation time used to prepare the extracts.

Results
WE43 samples were relatively cytocompatible, independent of size. The combination of WE43 with Zn1Mg led to galvanic corrosion with increased degradation of WE43 and slowed degradation of Zn1Mg, with some size-dependent cytotoxicity. Combination of WE43 with nitinol also led to galvanic corrosion, also with increased degradation of WE43. Nonetheless, the combination of WE43 with nitinol remained cytocompatible. The larger-sized Zn1Mg test probes showed substantial levels of cytotoxicity. PEO coating improved the cytocompatibility, as did decreasing the size (increasing the ratio of extraction medium to sample surface area). The combination of Zn1Mg with nitinol also led to galvanic corrosion, in this case with increased degradation of Zn1Mg and substantial levels of cytotoxicity. Evaluation of long-term cytocompatibility (up to 25 days) was achieved by refreshing the medium daily, as longer incubation times led to increased cytotoxicity of the extracts. Interestingly, these cytotoxicity results correlated well with ICP-OES results, identifying thresholds for toxicity for both magnesium and zinc. Further, zinc release from the Zn1Mg samples was found to be constant when the medium was refreshed daily whereas magnesium release from the WE43 samples was more variable.

Conclusions
Decreased size (surface area) leads to improved cytocompatibility, especially for the biodegradable metal alloys WE43 and Zn1Mg. Moreover, galvanic corrosion can strongly influence the degradation and resulting cytocompatibility when WE43, Zn1Mg, and/or nitinol are combined. These results suggest combinations of these alloys that can be used in the fabrication of multi-material implants and further identifies in vitro conditions that may predict toxicity in a mouse subcutaneous model.

Acknowledgement
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Topic: Tissue-Specific Focus
Subtopic: Brain
Type: Oral presentation

TERMIS25_1004 - Unlocking the potential of alginate biomaterial in advancing in vitro human brain organoid systems: exploring interactions and expanding applications

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Introduction/Objectives
This study aims to enhance current 3D *in vitro* brain models using alginate-based constructs. Human brain organoids represent a significant advancement over traditional 2D cell cultures and animal models, as they can closely replicate human neurodevelopmental and neuropathological processes, including cortical development and the growth of pediatric and adult brain tumors. However, organoids still face limitations: they fall short of capturing the full complexity and diversity of the human cortex, lack reproducibility, and do not include certain cell types, such as vasculature and microglia, which limits their capacity to accurately represent the brain's macro- and microenvironment.

Alginate-based hydrogels present a promising solution to address these challenges due to their controlled biochemistry and mechanical properties, biocompatibility, biodegradability, low cost, mild gelation process, and ease of handling and functionalization. Alginate microbeads were fabricated controlling size and mechanical properties, designed for use with hiPSCs-derived forebrain and cancer organoids. Further, these microbeads can act as carriers for region-specific morphogens or vascular cells, facilitating the development of multi-regional and vascularized cortical organoids. We herein show that the integration of alginate beads with cancer brain organoids could serve as an efficient platform for the targeted delivery of new drug molecules and develop personalized treatments.

Methods
An inkjet 3D printer was used for fabrication of sterile alginate-based microbeads (1% w/v), followed by cross-linking in either 0.1 M or 0.5 M $CaCl_2$ solution to produce SOFT and STIFF beads, respectively. Beads were co-cultured with hiPSCs-differentiating forebrain organoids from day 0 of differentiation for 100 days, and with patient-derived glioblastoma organoids for three months. At the end of the experiment, all conditions were fixed in PFA 4%, embedded in 5% agarose, and sectioned using a vibratome. Immunofluorescent (IF) staining was performed to assess changes in the expression of both mature (Tbr1, beta-3 tubulin) and proliferating (Ki-67, Nestin) brain cell markers.

Results
Alginate microbeads co-cultured with 3D cellular systems demonstrated positive integration and fusion with both hiPSCs-derived forebrain and glioblastoma organoids. Live imaging and whole-mount DAPI staining revealed the presence of alginate microbeads on the surface and within the core of brain organoids. IF analysis confirmed the successful integration of alginate microbeads within the organoid structure, without compromising its cellular composition.

Conclusions
Alginate microbeads shown a positive interaction with brain organoids. Based on these results, we are integrating human umbilical vein endothelial cells (HUVECs) within alginate microbeads to develop vascularized brain organoid models. This approach promises to enhance the structural and functional complexity of the organoids while reducing central necrosis. By introducing vascularization, we seek to establish a more accurate system to replicate human cortical development *in vitro* and to investigate the role of vasculature in brain diseases.

Topic: Submit to SYMPOSIUM
Subtopic: A Blueprint of Complex 3D Brain Architectures: Mechanics, Cell-Cell Interactions & Biomaterials Approaches
Type: Oral presentation

TERMIS25_1013 - Mechanical properties of engineered matrices modulate microglial activation and inflammatory responses

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Introduction/Objectives
Neurodegenerative disorders pose a significant socioeconomic burden, a challenge expected to grow with increasing life expectancy. Microglial cells, as primary immune responders within the central nervous system (CNS), play a critical role in the brain's immune response and, consequently, they are prime players in the context of CNS neurodegenerative diseases. Recent research has linked their function to mechanotransduction processes. In this study, we present a novel 3D tissue-engineered platform to further investigate these dynamics.

Methods
Primary rat microglia were encapsulated in alginate hydrogels containing cell adhesion motifs and matrix metalloproteinase-sensitive peptides to simulate the extracellular matrix (ECM) environment. We produced varied matrices with different alginate concentration, characterizing their mechanical properties using bulk rheology. A pro-inflammatory stimulus was applied, and microglial metabolic activity, nitrite production, and gene expression levels were measured. Additionally, image analysis was conducted to assess microglial morphology within the alginate matrices.

Results
Microglial metabolic activity remained stable across different alginate formulations, either in the presence or absence of a lipopolysaccharide (LPS) stimulus. However, image analysis revealed an increase in cell volume and a decrease in sphericity in response to LPS across all formulations. Furthermore, in the presence of LPS, microglia in 1% (w/v) alginate showed increased nitrite production and higher expression of NOS2 and IL-6, consistent with a pro-inflammatory response. Interestingly, 2% (w/v) alginate formulations (stiffer) were associated with reduced TLR4 expression, suggesting a potential regulatory mechanism.

Conclusions
Our findings indicate that microglial response to inflammatory stimuli is influenced by the mechanical properties of the surrounding matrix. This work provides insights that may aid in identifying therapeutic targets for managing neuroinflammation and, ultimately, neurodegenerative diseases.

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Topic: Submit to SYMPOSIUM
Subtopic: A Blueprint of Complex 3D Brain Architectures: Mechanics, Cell-Cell Interactions & Biomaterials Approaches
Type: Oral presentation

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TERMIS25_1014 - Novel 3D astroglia models: Studying the mechanobiology of astrocytes in health and disease

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Introduction/Objectives
The extracellular matrix (ECM) biomechanical cues have recently been recognised as possible mediators of neurologic diseases' onset and progression. Astrocytes respond to an insult with changes in gene and protein expression profiles and are additionally key mediators of matrix remodelling in the central nervous system (CNS). The above makes them promising targets to tackle pathology. However, the role of the ECM and its mechanical properties in influencing astrocyte behaviour in health and disease is not yet understood. Here we use a novel 3D in vitro model to dissect astrocyte behaviour in response to changes in the mechanical properties of the microenvironment under pathological conditions.

Methods
Primary rat astrocytes were encapsulated in modified alginate hydrogels containing the cell adhesive peptide RGD, and the matrix metalloproteinase sensitive peptide PVGLIG, on partially oxidized alginate. Matrices with different mechanical properties were produced by changing the polymer mass (1% and 2% wt/v) as well as the ratio of oxidized to non-oxidized alginate (60:40, 30:70) and were characterized by bulk rheology. Cell response was assessed via metabolic activity, immunostaining for cytoskeleton markers and gene expression analysis. To emulate an insult, cells were subjected to oxygen and glucose deprivation (OGD; <1% O2) for 24h, followed by transition to normoxia and complete media. The cell response was evaluated by metabolic activity and lactate dehydrogenase (LDH) release assessment, immunofluorescence for cytoskeleton markers and quantification of gene expression for astrogliosis marker genes, at different timepoints after oxygenation and nutrition reestablishment.

Results
Changing the polymer mass and the ratio of oxidized to non-oxidized alginate resulted in hydrogels with different mechanical properties. Astrocytes remain metabolically active in all hydrogel formulations but present different morphologies depending on the matrix mechanical properties. Gene expression analysis showed a general upregulation of astrocytic markers in the 1% (wt/v) matrices were cells presented the most complex, ramified morphologies. 24h of OGD significantly decreased the metabolic activity of cells embedded in modified alginate while increased LDH release. Image analysis showed differences in GFAP staining volume and sphericity between control and OGD conditions and different reoxygenation times.

Conclusions
The astrocytic phenotype was explored in relation to the environment mechanical properties and OGD was successfully implemented in a 3D in vitro glial tissue engineered hydrogel-based system. This work provides a realistic and versatile system for studying cellular phenotypes in optimized microenvironments and in response to different stimuli.

Acknowledgments
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Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1121 - Investigating the effects of hydrostatic pressure on neural cells in a novel in vitro model of traumatic brain injury

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Introduction/Objectives
The relationship between raised intracranial pressure (ICP) and mortality in traumatic brain injury (TBI) patients is well-established; however, *in vitro* investigations into this phenomenon are scarce. This study aims to model and investigate the effects of hypoxia and raised ICP on neural cells following TBI using a novel *in vitro* model of TBI within customised, pressurisable microfluidic devices.

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Methods
Microfluidic devices were fabricated via standard soft lithography techniques. Neuron-like cells (NLCs) were generated by partially differentiating N2a cells for 48 hours, then co-cultured within microfluidic devices in combination with astrocyte (C8-D30) and/or microglia (BV2) cell lines. Cells were seeded in approximate ratios of 75:25:5 NLCs:astrocytes:microglia in triple co-culture and 95:5 NLCs:microglia for these double co-culture devices. Following overnight attachment, syringe pumps containing differentiation medium (HG DMEM + 2% FBS + 20 μM retinoic acid) or hypoxic medium (differentiation medium + 1000 μM $CoCl_2$) were connected to devices for 24 hours for maintenance. 40 mmHg hydrostatic pressure was then applied to pressure groups for 1-1.5 hours. All devices were connected to fresh differentiation medium supply for 6 hours, followed by characterisation via live/dead assay, imaging (n≥3 imaging regions per device) and analysis using CellProfiler.

Results
Survival (mean % ± SEM) (n=number of devices) of NLCs + astrocytes co-culture exposed to hypoxia + pressure (47% ± 0.02) (n=3) was lower than survival of cells exposed to hypoxia alone (55% ± 0.02) (n=3). Similarly, survival of NLCs co-cultured with microglia exposed to hypoxia + pressure (34% ± 0.01) (n=3) was lower than cells exposed to hypoxia alone (40% ± 0.01) (n=3). Survival was not significantly different between hypoxia only (59% ± 0.02) (n=2) and hypoxia + pressure (58% ± 0.03) (n=2) in the NLC + astrocytes + microglia devices. Average survival in control devices for each cell grouping was above 85%, as was the average survival of triple co-cultured cells exposed to pressure alone. Comparing co-cultured NLCs + astrocytes with NLCs + astrocytes + microglia via Mann-Whitney tests showed significant differences between hypoxia + pressure groups of each cell grouping ($p=0.0072$) while the difference in survival of these cell groups exposed to hypoxia only was not significant ($p=0.0788$). A similar comparison of NLCs + microglia with NLCs + astrocytes + microglia showed significant differences between the hypoxia + pressure ($p<0.0001$) and the hypoxia only ($p<0.0001$) groups for these cell groupings.

Conclusions
These data suggest that neural cells may be damaged by raised hydrostatic pressure when this pressure is combined with hypoxia. Initial data indicate that astrocytes may play an important role in regulating cell survival following insult by hypoxia and/or hydrostatic pressure, and that there exist interactions between microglia and astrocytes responsible for conferring neuronal protection from combined hypoxia/pressure insult. These experiments could improve our understanding of the cellular events underlying poor outcomes for patients who experience raised ICP following TBI.

Topic: Gene therapy
Subtopic: Gene delivery
Type: Oral presentation

TERMIS25_212 - Precision nanomedicine for targeting cardiomyocytes to enhance cardiac regeneration through microRNA delivery

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Introduction/Objectives
The irreversible loss of cardiomyocytes (CMs) caused by myocardial infarction (MI) may lead to heart failure [1]. Only fetal or neonatal CMs from newborn mammals show proliferative ability, while little or no proliferative capacity has been observed in adult CMs [2]. Recently, it has been demonstrated that viral-mediated expression of exogenous microRNAs (miRNAs), such as miR-199-3p, can promote the re-entry of resident neonatal and adult CMs into the cell cycle, stimulating their proliferation [3]. The *in situ* release of miRNAs may represent a promising solution for restoring cardiac function. However, its translation to clinics requires nanocarriers as safer and more efficient alternatives to viral vectors [4]. In this work, novel hybrid polymer-lipid nanoparticles (NF-NPs), surface-functionalized with a peptide (F-NPs), were developed to efficiently release miR-199-3p to target CMs, minimizing off-target effects.

Methods
NPs, loaded with miR-199-3p, control negmiR, or siRNA-Cy5, were formulated based on a patented composition and method. F-NPs were decorated with a cardiac specific peptide, which selectively recognizes CMs, especially under hypoxic conditions. The peptide functionalization degree was optimized to maximize cellular uptake. NF-NPs and F-NPs were characterized for their physicochemical properties. Their cytocompatibility, uptake, and transfection efficiency were evaluated using H9c2 cell line, with Lipofectamine RNAiMAX, a lipid commercial transfection agent, serving as control. *In vitro* cell tests with H9c2 were conducted under both normoxic and hypoxic conditions (100 μM hydrogen peroxide (H_2O_2) for 1 h). Further tests were conducted using induced pluripotent stem cell-derived CMs (iPSC-CMs) and human cardiac fibroblasts (CFs) in 2D cultures and *in vitro* models.

Results
NF-NPs and F-NPs showed an average hydrodynamic diameter of 150-250 nm, a negative Z-potential, complete miRNA encapsulation (99 %), controlled miRNA release in 9 days, and storage stability up to 15 days. NPs showed high cytocompatibility in both normoxic and hypoxic conditions and high uptake by H9c2. Selective uptake by iPSC-CMs over CFs and transfection efficiency of F-NPs was shown in 2D cell cultures and in *in vitro* models of human cardiac fibrotic tissue, obtained by co-culturing iPSC-CMs and CFs on biomimetic scaffolds [5].

Conclusions
F-NPs showed cytocompatibility, cell uptake and transfection efficiencies comparable to Lipofectamine RNAiMAX. Unlike commercial agents, F-NPs showed promise for future *in vivo* applications, due to their long-term storage stability and target efficiency demonstrated in relevant *in vitro* human cardiac tissue models. *In vivo* trials in infarcted mouse model, planned for early 2025, will provide further evidence on F-NPs efficiency to release miRNAs to target CMs. PoliRNA Srl, a spin-off of Politecnico di Torino, was founded with the aim of translating F-NP technology to the market.

Acknowledgements
This project has received funding from the European Research Council (ERC) under the European Union's Horizon research and innovation program grant agreement No 101113522 (POLIRNA project).

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Topic: Gene therapy
Subtopic: Biomaterial-guided gene therapy
Type: Oral presentation

TERMIS25_711 - A multi-faceted gene-activated scaffold as an immuno-modulatory platform for chronic wound healing applications

Juan Carlos Palomeque[1 2], Matthew McGrath[1 2], Marko Dobricic[1], Cian O'connor[1], Cathal Kearney[3], Shane Browne[1 4], Fergal O'brien[1 2]

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Introduction/Objectives
Macrophage polarization plays an essential role in wound healing. The initial inflammatory response is facilitated by M1 macrophages,

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whereas the subsequent resolution of inflammation and promotion of vascularization are mediated by M2 macrophages. However, this balance is dysregulated at a genetic level in chronic wounds, increasing the M1 macrophage presence, enhancing inflammation, and reducing vascularization. While collagen-based (CG) scaffolds show promise for wound repair, additional functionalization is required to overcome this dysregulation and elicit chronic wound healing.[1] microRNAs (miR) regulate gene expression in many biological processes, including inflammation and angiogenesis in wound healing.[2] miR-155 inhibition, in particular, has shown anti-inflammatory outcomes in vivo, while increasing pro-angiogenic marker expression.[3] Thus, we propose that targeted delivery of miR-155 inhibitor from CG scaffolds to macrophages will promote M2 polarization, effectively attenuating inflammation. Additionally, we assessed the interaction between miR-155-treated macrophages and endothelial cells, and their ability to enhance angiogenic processes necessary for chronic wound healing.

Methods
Nanoparticles were synthesized by complexation of miR-155 and non-viral GAG-binding enhanced transduction (GET) peptide.[4] miRNA-activated scaffolds were formed by soak-loading miR nanoparticles onto lyophilized CG scaffolds. The effect of miR delivery on macrophage polarization towards non-polarized (M0) and pro-inflammatory (M1) phenotypes was assessed using THP-1 monocytic cells. Cell phenotype and polarization was assessed by confocal microscopy through staining and quantification of pro-inflammatory (CD80) and anti-inflammatory (CD206) markers. The effect of macrophage polarization on angiogenesis was evaluated through macrophage-endothelial cell interactions via paracrine signaling in migration and tube formation assays. Analysis of the expression of key inflammatory and angiogenic markers, including TNF-α, IL-10 and VEGF, was examined by PCR and ELISA.

Results
miRNA-activated CG scaffolds increased the expression of anti-inflammatory and angiogenic markers, IL-10 and VEGF, from M0 and M1 macrophages over 7 days. Increased CD206+ and reduced CD80+ macrophage numbers were observed on M0 and M1-seeded scaffolds, respectively. Similarly, reduced circularity was quantified from M0 and M1 macrophages on miRNA-activated scaffolds with greater circularity indicating a pro-inflammatory phenotype. Endothelial cell migration and vascular-like structure organization was enhanced when exposed to the conditioned media from miRNA-activated scaffolds.

Conclusions
In this work, we show that miRNA-activated CG scaffolds enhance an anti-inflammatory M2 phenotype confirmed through gene expression and the quantification of CD206+ and CD80+ expression. We show that the secretome from these macrophage-seeded miRNA-activated scaffolds enhance pro-angiogenic processes in endothelial cells, essential for the vascularization of chronic wounds. Taken together, this data indicates that miRNA-activated scaffolds enable M2 macrophage polarization; key for inflammation reduction, promotion of angiogenesis, and the resolution of chronic wounds.

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Topic: Gene therapy
Subtopic: Biomaterial-guided gene therapy
Type: Oral presentation

TERMIS25_1217 - Immune cell-membrane derived vesicles as smart nanovectors for gene delivery

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Introduction/Objectives
Nowadays, the new frontier of cancer therapy is represented by immunotherapies which include several approaches exploiting the immune system's ability to target, and attack cancer cells. In particular, cancer immunotherapy based on engineered T cells has emerged as an effective tool in the treatment of cancer. In this frame, one of the main goals of nanomedicine for the advancements of immunotherapy is focused on the generation of smart nanovectors to achieve therapeutic gene delivery in patient's immune cells, thus conferring to the engineered cells the ability to fight against patient-specific cancer. However, the design of these smart nanovectors required to adopt innovative strategies able to confer enhanced targeting properties, thus considering the presence of biological barriers such as tumor heterogeneity, clearance by immune system, osmotic pressure, and stromal microenvironment. In this scenario, this work was focused on the development of cell-membrane derived nanovesicles by exploiting their ability to mimic both biological and functional features of the native cells, thus ensuring to nanoparticles self-recognition, immune system evasion and long-time circulation *in vivo*.

Methods
To this aim, immune cells were collected and subjected to first hypotonic cell lysis and differential centrifugation for purification of plasma membranes. Cell plasma membranes were subjected to the extrusion method by using nanoporous membrane filter for the nanovesicle's synthesis. Dot blot, dynamic light scattering analysis (DLS) and transmission electron microscopy (TEM) were useful techniques for evaluating the surface protein's retention, the particle's average size and the morphology structure. To examine the cellular uptake, cell membrane derived nanovesicles were co-incubated with pre-seeded immune cells for different time points and analysed by using flow cytometry and confocal fluorescence microscopy.

Results
Physical characterizations confirmed the successful production of monodispersed immune derived vesicles in the nanometric size range. Dot blot analysis confirmed the retention of protein exposed on nanovesicle's surface. TEM analysis revealed the core-shell structure as expected in a lipid bilayer-coated particle. Data obtained from flow cytometry showed a high internalization rate of synthetized nanovesicles withing immune source cells. The cellular uptake was further confirmed by confocal microscopy.

Conclusions
The well-shaped and nanometric immune derived nanovesicles retained lipid molecules and proteins on their surface which allowed them the homotypic targeting and the high cellular uptake within source cells. The further step is focused on the optimized production of nanovesicles encapsulating plasmid DNA with the aim to efficiently engineer T cells to be employed for targeted immune cancer therapy.

Topic: Gene therapy
Subtopic: Gene delivery
Type: Oral presentation

TERMIS25_1401 - Development of Functionalized Polymer-Lipid Hybrid Nanoparticles for Skeletal Muscle-Targeted RNA Delivery in Spinal and Bulbar Muscular Atrophy (SBMA) Therapy

Giovanni Paolo Stola[1 2 3], Letizia Nicoletti[1 2 3], Martina Coletto[1 2 3], Martina De Carolis[1 3], Camilla Paoletti[1 2 3], Roberta Andreotti[4 5], Elena Marcello[1 2 3], Clara Mattu[1 2 3], Manuela Basso[6], Maria Pennuto[4 5], Valeria Chiono[1 2 3]

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Introduction/Objectives
Spinal and bulbar muscular atrophy (SBMA) is a rare X-linked disorder caused by mutations in the androgen receptor gene, and characterized by progressive muscle weakness, wasting, and spasms, significantly reducing patient quality of life [1]. Current therapeutic strategies are limited, necessitating the development of targeted and effective treatments. Recently, artificial microRNAs (amiRs) were designed to downregulate overexpressed genes implicated in SBMA pathogenesis, such as lysine-specific demethylase 1 and protein arginine N-methyltransferase 6 [2]. Initial studies have demonstrated that amiRs improve motor function in SBMA murine models [2]. However, amiRs were delivered using viral vectors, which poses safety concerns and limits clinical translation. To address these challenges, we designed and patented polymer-lipid hybrid nanoparticles (NPs) as a promising miRNA delivery platform with high biocompatibility, high loading efficiency, and potential for surface functionalization. This study focuses on developing polymer-lipid hybrid NPs functionalized with a skeletal muscle-specific peptide (MSP) for targeted amiRs delivery to skeletal muscle cells.

Methods
Polymer-lipid hybrid NPs functionalized with MSP were prepared based on a patented method. Optimization of the functionalization involved conjugating MSP to polyethylene glycol spacer (MSP-PEG), followed by MSP-PEG grafting to NP surface via click chemistry (PEG-MSP NPs). The aim was to enhance peptide exposure and cellular uptake. The nanoparticles were characterized for hydrodynamic size, zeta potential, and functionalization efficiency. *In vitro* internalization studies were performed using C2C12 murine myoblasts and differentiated myotubes, comparing PEG-MSP NPs with non-functionalized NPs through fluorescence microscopy and flow cytometry. Dose-dependent cell viability assays were also performed.

Results
PEG-MSP NPs showed a hydrodynamic size of ~200 nm, a zeta potential of -18.7 mV, and 80% functionalization efficiency. In C2C12 myoblasts, PEG-MSP NPs exhibited high internalization (99.2% Cy5+ cells; 12,925 RFU fluorescence intensity). Higher internalization of PEG-MSP NPs versus non-functionalized NPs was confirmed in tests with differentiated myotubes, better reproducing physiologically relevant conditions. High cell viability (>90%) was found for all tested concentrations.

Conclusions
Results showed that PEG-MSP NPs represent a promising platform for RNA-based therapies, demonstrating superior delivery efficiency, high biocompatibility, and potential for targeted delivery to skeletal muscle cells. Future studies will focus on evaluating therapeutic efficacy of PEG-MSP NPs/amiRs *in vitro* using human myoblasts and *in vivo* in SBMA murine models, alongside their tissue biodistribution. In conclusion PEG-MSP NPs represent a safe and effective non-viral miRNA delivery system with the potentiality of transforming the treatment of SBMA and other skeletal muscle disorders.
Acknowledgments: This project is supported by ERC BIORECAR-EU H2020 GA 772168, ERC-2022-PoC2 POLIRNA GA 101113522 and AFM TELETHON SaveAR.

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Topic: Cells
Subtopic: Cell signaling
Type: Oral presentation

TERMIS25_1481 - Red Light Activated TGFB Signalling to Drive hPSC Chondrogenesis

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Introduction/Objectives
Osteoarthritis affects 7.6% of the global population and leads to articular cartilage defects.[1] To generate cartilage tissue analogues for defect site regeneration, chondrogenesis protocols direct human pluripotent stem cells (hPSCs) towards permanent articular chondrocytes *in vitro*. Activation of transforming growth factor beta (TGFB) family signalling at precise timepoints during differentiation is imperative to prevent deviation of cells to off-target lineages. Expensive and batch-variable growth factors provide limited control over cell signalling, particularly within 3D biomaterial scaffolds, and so achieving a stable articular chondrocyte phenotype remains a challenge. Optogenetic systems harness light to dynamically activate signalling pathways with spatial and temporal precision. Already, blue light activation of TGFB family signalling has been used to drive hPSC chondrogenesis, and shown potential to create 3D signalling gradients.[2-5] However, all systems are blue light activated. This limits clinical applicability due to photoxicity concerns, may interfere with photocrosslinking of biomaterial scaffolds, and means only one signalling pathway can be controlled at a time. To exploit the potential of optogenetics to fine-tune chondrogenic phenotype, this work aims to add an additional activation wavelength to the optogenetic toolkit.

Methods
We designed a red light activatable TGFB signalling system using PhyB and Pif photosensitive proteins coupled to the cytoplasmic domains of native TGFB receptors. Lentiviral vectors were constructed to engineer the system into a human chondrogenic cell line (TC28a2) , which contained a bioluminescent reporter for TGFB pathway activation.[5] To test activation, engineered cells were incubated in serum free medium, containing 1μM phycocyanobilin (PCB) for PhyB activation, and stimulated for 3 hours with 10ng/ml TGFB3 or 630nm light (3.4mW/cm^2). Cells were then imaged in an Alligator Luminescence System and expression of selected TGFB response genes was measured using RT-qPCR.

Results
RT-qPCR detection of engineered transgenes and confocal imaging of fluorophore tags confirmed expression of optogenetic TGFB receptors in TC28a2 cells. Compared to non-stimulated cells, TGFB and red light stimulated cells had strong bioluminescent signals, corresponding to TGFB activity, and upregulated TGFB response gene expression.

Conclusions
The results indicate that optogenetic TGFB receptors were successfully expressed in TC28a2 cells. Detection of TGFB signalling activity following red light stimulation suggests that it can effectively induce dimerization of optogenetic receptors to switch on TGFB signalling. This is evidence that we have, for the first time, engineered red light control of the TGFB signalling pathway in a human cell line, which may be more suitable for cartilage tissue engineering than existing blue light optogenetic systems. Future application in hPSC chondrogenesis could open opportunities for controlling multiple signalling pathways with different light wavelengths to better guide formation of a stable chondrogenic phenotype within cartilage tissue analogues.
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Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_917 - Electroactive polysaccharide-based click-hydrogels: targeting stimuli responsive biomaterials for skin wound healing

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Introduction/Objectives
The main function of skin, which is the largest and most exposed organ of the human body, is to protect us against environmental threats and pathogens. However, skin is also vulnerable to trauma or diseases, with a wound repair process that is complex and slow.[1] Hence, within this context, our goal is to promote the healing of challenging skin wounds by combining conductive wound dressings composed of polysaccharide-based click-hydrogels and electrical stimulation (ES), thus following an advanced cost-effective approach. Not only that, but we also aim at producing multifunctional biomaterials, with our hydrogel scaffolds potentially serving as drug delivery platforms, as well as sensor devices.

Methods
Click-hydrogels, made of hyaluronic acid (HA) or alginate (Alg), were produced by an efficient nucleophilic thiol-yne addition using polyethylene glycol (PEG) as cross-linker. All precursors were modified with thiol or alkyne moieties following previously published procedures.[2] After an optimization process, polysaccharide-based click hydrogels were prepared adjusting the total solid content and the weight ratio of the precursors, which resulted in robust and non-swellable hydrogels with enough internal cohesion for further handling, characterization and, most importantly, application in a biological setting. Later, we rendered them electroactive by semi-interpenetrating a conducting polymer, poly(hydroxymethyl-3,4-ethylenedioxythiophene) (PEDOT-MeOH), prepared by oxidative polymerization. The chemical and structural properties of the resulting hydrogels, as well as their mechanical and electrochemical performance were characterized by several techniques. Besides, cell viability assays were conducted to verify their cytocompatibility, while an *in vitro* ES test was performed to simulate the healing process of a skin wound.

Results
For both polysaccharides, mechanically stable systems were obtained with a gelation time of 1 minute, while gelation fraction values were determined to be between 71 ± 1% and 88 ± 4%. The presence of PEDOT-MeOH within the click-hydrogel was confirmed by X-ray photoelectron spectroscopy, while a honeycomb-like morphology with PEDOT-MeOH covering the pore walls was observed for the conductive hydrogels. Although PEDOT-MeOH did not influence the water absorption capacity of blank click-hydrogels, they affected their mechanical (*i.e.* Young's modulus and compressive strength) and electrochemical performance. Finally, regarding the potential for skin regeneration of our systems, it was verified that the conducting hydrogel formulation is cytocompatible, with cell viability values higher than 80%. Most importantly, it promoted efficient cell migration by ES using small voltages for short periods. Indeed, in just 1 h following 15 min of ES, the wound gap created was repaired with a homogeneous monolayer of migrated epithelial cells.

Conclusions
Overall, we have demonstrated that the combination of conducting polysaccharide/PEDOT-MeOH click-hydrogels and ES is a promising system for skin tissue regeneration. In subsequent studies, we aim at using 3D printed skin to evaluate the proliferation, migration and angiogenesis of cells in a more complex and biorelevant configuration.

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Topic: Submit to SYMPOSIUM
Subtopic: Advancements in Electroactive Biomaterials for Cell Fate Modulation and Tissue Regeneration
Type: Oral presentation

TERMIS25_948 - Laser-based conductive biomaterials for flexible bioelectronics

Carlos Ureña Martín[1], Beatriz MacHado[2], Vasco Teixeira[2], Maria Morais[2], Tomás Pinheiro[2], José M. Inácio[3], Melanie Hart[4], Elvira Fortunato[2], Rui Igreja[2], Henrique Almeida[2]

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Introduction/Objectives
This study investigates laser-induced graphene (LIG) formation on lignin-agarose membranes using both CO_2 and UV lasers. By leveraging lignin's biocompatibility and LIG's conductive properties, we aimed to create bioelectronic interfaces suitable for applications like cardiac tissue stimulation.

Methods
Lignin-agarose membranes were fabricated with varying lignin concentrations and separately treated with CO_2 and UV laser processes to generate LIG. The CO_2 laser was applied to achieve broad, large-area graphitization, while the UV laser provided higher resolution and controlled thermal effects, minimizing substrate damage. Characterization techniques, including Raman spectroscopy, SEM, and XPS, were used to confirm graphene formation and assess structural integrity.

Results
Both CO_2 and UV laser treatments successfully generated LIG on lignin-agarose membranes, each method offering distinct benefits. The CO_2 laser facilitated consistent and scalable graphitization, suitable for creating flexible bioelectronic surfaces. The UV laser, with its refined resolution and minimal thermal impact, produced LIG with enhanced conductivity and stability, supporting cardiomyocyte alignment and responsiveness to electrostimulation.

Conclusions
The use of both CO_2 and UV lasers for LIG formation on lignin-based membranes demonstrates a versatile approach to developing conductive, biocompatible materials for bioelectronics.

Topic: Submit to SYMPOSIUM
Subtopic: Advancements in Electroactive Biomaterials for Cell Fate Modulation and Tissue Regeneration
Type: Oral presentation

TERMIS25_1290 - Bio-orthogonal electroconductive gelatin-based hydrogels as biomimetic substrates for in vitro cardiac tissue modeling

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Introduction/Objectives
Hydrogels for the in vitro engineering of cardiac tissues developed so far have generally failed to provide the intricate interplay of electrical, mechanical, and biochemical cues needed for cell functional maturation. Electroconductive hydrogels (ECHs) have demonstrated

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their potential to enhance electrical cell-cell synchronization. However they should also provide biomimetic composition and mechanical properties to support in vitro cardiac tissue maturation. Previously we have designed gelatin-based photo-curable bioactive ECHs [1]. However, radical-initiated chain-growth polymerization has limitations such as unreacted double bonds and network heterogeneities.

The aim of this work was to design gelatin-based ECHs by bio-orthogonal step-growth click reactions. The addition of ionic cross-linking and an electroconductive filler were investigated to get tailored electroconductivity, bioactivity and biomimetic viscoelastic properties with cardiac tissue-like stress-relaxation behavior.

Methods
Double cross-linked alginate-gelatin hydrogels were developed using bio-orthogonal strain-promoted alkyne-azide cycloaddition (SPAAC) click chemistry and ionic cross-linking (AG hydrogels). Alginate and gelatin were functionalized with azide groups. Dibenzocyclooctine (DBCO) was used as cross-linker with/without further calcium ion crosslinking of alginate chains. Titanium carbide MXene quantum dots (MQDs) were incorporated into AG hydrogels to enhance their electroconductivity. Hydrogels were characterized for their viscoelastic, electrical, and biological properties, including cell viability, adhesion, and expression of cardiac-specific markers in human cardiac fibroblasts (HCFs) and cardiomyocytes (CMs).

Results
Bio-orthogonal AG hydrogels were successfully developed by SPAAC click reaction, while double crosslinked AG hydrogels were obtained by further calcium ion crosslinking. Viscoelastic properties of hydrogels were tuned by chemical and ionic crosslinking degree, reaching cardiac tissue-like stress-relaxation behavior. MQDs dispersion into the hydrogels enhanced their electroconductivity without altering their viscoelastic behavior. Hydrogels were found to be biocompatible for HCFs and CMs, supporting their adhesion. Substrate viscoelasticity regulated HCF behavior and promoted the maintenance of a physiological phenotype, inhibiting activation into myofibroblasts, while MQDs addition promoted increased expression of gap junctional protein connexin-43 in CMs.

Conclusions
Novel bio-orthogonal AG-based ECHs were designed for cardiac tissue engineering obtaining biomimetic viscoelasticity resembling dynamic mechanical properties of native myocardium. These substrates represent a promising platform to obtain functional in vitro cardiac models.

Acknowledgements
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Topic: Submit to SYMPOSIUM
Subtopic: Advancements in Electroactive Biomaterials for Cell Fate Modulation and Tissue Regeneration
Type: Oral presentation

TERMIS25_1459 - Wireless Nanobioelectronic Systems to Modulate Breast Cancer Bioelectricity

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Introduction/Objectives
Cancer cells exhibit unique bioelectrical properties that influence their tumorigenic potential and therapeutic strategies targeting these remain underexplored. Here, a wireless nanobioelectronic system to modulate cancer cell bioelectricity based on piezoelectric and conductive nanoparticles (BTO@PEDOT NPs) was developed and activated by ultrasound (US) *in vitro* on two human breast cancer cell lines (MCF-7, MDA-MB-231) and healthy fibroblasts (HMF), and validated their efficacy *in vivo* using the 4T1 murine breast cancer model.

Methods
BTO@PEDOT NPs consisted of a barium titanate (BTO) core and poly(3,4-ethylenedioxythiophene) (PEDOT) shell. Physico-chemical characterisation and biocompatibility of these was performed. Cells were treated with 200 μg/mL of BTO@PEDOT NPd and stimulated by US. Intracellular reactive oxygen species (ROS), calcium levels and cell membrane potential were measured. For *in vivo* studies, 4T1 cells were injected into the mammary gland of C57BL/6 mice, divided into three groups: control, NPs, and NPs + US. 100 μl of NPs (600 μg/ml) were injected every 2 days starting day 7 and US was applied at the tumor site at an intensity of 0.4 W/cm^2 for 150 sec a cycle, (3 cycles at day 7; 2 cycles/day from day 8 to 12). Tumor growth was monitored until day 15, followed by postmortem analysis.

Results
Following US stimulation, cell viability decreased significantly in breast cancer cells treated with BTO@PEDOT NPs, reducing to 31% (MCF-7) and 24% (MDA-MB-231), while HMF cells remained unaffected. Mechanistic studies revealed that nanoparticle activation increased ROS and intracellular calcium concentrations, disrupting the cancer cells' bioelectronic homeostasis. Membrane polarisation halted cell cycle progression in cancer cells, while HMF polarisation did not affect viability nor cell cycle, suggesting a direct interference with their tumorigenic circuitry. *In vivo*, the NPs + US group showed remarkable efficacy in inhibiting tumor growth in the 4T1 murine breast cancer model. Other test groups showed no significant differences, confirming the synergistic efficacy of the combined treatment.

Conclusions
BTO@PEDOT NPs activated by US can selectively modulate the bioelectricity of breast cancer cells without affecting healthy cells. The system's ability to polarise cancer cell membranes and halt their proliferation was further validated in an aggressive *in vivo* murine model, where tumor growth was significantly inhibited by the NP+US treatment. These findings highlight bioelectricity as a critical target for future cancer interventions, paving the way for innovative strategies to combat tumor progression.

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Type: Oral presentation

TERMIS25_157 - Integrating mineralization with tomographic volumetric bioprinting for fast photofabrication of bone-like constructs

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Introduction
In vitro 3D bone models often lack the embedding of osteocyte-like cells within a bone-mimetic matrix that also replicates the native trabecular architecture. This is especially required for future models in which bone remodeling and adaptation can be linked to osteocyte functionality. Tomographic volumetric bioprinting (VBP) enables the creation of complex, cell-laden constructs through tomographic light projections, offering superior speed and design freedom compared to traditional layer-by-layer methods. However, integrating both high cell densities and mineral components into photocurable resins poses significant light scattering challenges, which can compromise print quality. In this study, we aimed at developing a cytocompatible methodology to control mineral crystallization within gelatin methacryloyl (GelMA) bioresins, with the goal to reduce mineral particle-induced light scattering. We combined this method with matching the refractive index (RI) of cells and resin, to further decrease light scattering and allow fabrication of mineralized hydrogel constructs at a more physiologically relevant cell density.

Methods
Mineral crystallization was controlled using the polymer-induced liquid-phase precursor (PILP) mechanism, which stabilizes amorphous calcium phosphate (ACP) in supersaturated solutions using poly-aspartic acid (pAsp). GelMA (5%) resins containing 0.1% Lithium-Phenyl-2,4,6-trimethylbenzoylphosphinat were supplemented with salts (9 mM $CaCl_2$ and 4.2 mM K_2HPO_4) and optionally 1 mg/ml pAsp. Resins were evaluated (N=3) for mineralization, light transmission, and printability using scanning electron microscopy (SEM), orthogonal light projections, and VBP, respectively. To enable VBP of cell- and ACP-containing constructs, resin RI was increased to the range of the cell RI by mixing 10% iodixanol into the resins. Trabecular bone-like constructs were printed with a human mesenchymal stromal cell (MSC) density of 3 million cells/ml in the presence and absence of pAsp-stabilized ACP. The influence of ACP on cell viability and differentiation was subsequently evaluated following a 28-days osteogenic culture (N=4), using live/dead and immunohistochemistry (IHC) for osteocalcin and podoplanin.

Results
In GelMA resins with salts but without pAsp, plate-like mineral crystals formed, while the addition of pAsp resulted in more amorphous minerals. Light transmission significantly decreased in the GelMA + salts resins compared to salt-free resins ($p<0.05$) but was partly restored with pAsp ($p<0.05$). These results translated into improved printability for salt and pAsp containing resins. Constructs with only salts always had features that were over-polymerized and/or under-polymerized. MSC-laden trabecular-like constructs demonstrated excellent cell viability of 94.9 ± 2.8% in GelMA and 94.2 ± 2.2% in GelMA + ACP (*ns*) one day post-VBP. After 14 and 28 days, positive IHC signals were observed for osteocalcin and podoplanin in GelMA constructs with pAsp-ACP, which was not observed in GelMA constructs.

Conclusions
By controlling mineral crystallization in GelMA bioresins and combining it with RI matching and VBP, bone-like constructs with a mineralized matrix were printed within 40 seconds at a cell density of 3 million cells/ml. Within these constructs, MSCs showed signs of osteogenic differentiation. Therefore, this methodology has the potential to advance the development of *in vitro* bone models.

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Type: Oral presentation

TERMIS25_244 - Iodixanol enhances xolographic reaction rates enabling cytocompatible and high-resolution volumetric bioprinting of living matter

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Introduction/Objectives
The engineering of functional living tissues with multiscale and hierarchical features has remained a key, largely unsolved challenge. Conventional bioprinting approaches have shown promise in their ability to realize such features, but are unable to achieve this at a clinically relevant time scale.[1] Lately, rapid volumetric bioprinting has been invented to overcome this technical hurdle. Xolography is a recently developed volumetric printing technique that does indeed print plastic resins at both high speed and resolution. [2] However, xolography currently demands the use of high concentrations of cytotoxic co-initiators, such as triethanol amine (TEA), to achieve fast, high-resolution printing. This essential reliance on cytotoxic compounds currently forces xolographic bioprinting to choose between fast print speeds or cytocompatibility. Here, we report that iodixanol (IDX) improves xolographic print reactivity allowing for the near-elimination of cytotoxic co-initiators, enabling rapid, yet cytocompatible xolographic bioprinting. As an additional benefit, IDX reduces cell-mediated light scattering by matching the refractive index (RI) of the print bath to the cells contained within, improving bioprint resolution. Together, we demonstrate that the use of IDX allows for rapid, cytocompatible, and high-resolution xolographic bioprinting of living matter.

Methods
To identify the optimal IDX concentration for high-resolution printing, we prepared GelMA-based bioinks and incorporated 3T3 fibroblasts at a concentration of 10 M/mL. Visible light transmission was measured for various IDX concentrations to determine the lowest scattering inks. The cell-gel RI-matching was then verified by holotomography. To determine resolution, these cell-containing inks were then printed. Dual-color photorheology was used to determine xolographic print reactivities. Cell viability was analyzed using various fluorescent and biochemical assays. Print resolutions were investigated using holotomography, confocal microscopy, and Schlieren imaging, as well as by printing various complex shapes within minutes.

Results
Optimal bioink transmission was achieved at 24% IDX. Holotomography verified that increased light transmission resulted from the matching of print bath RI to cytoplasm RI. Dual-color photorheology revealed that print reactivity increased with IDX in a dose-dependent manner. Notably, the reactivity increased more at lower amounts of TEA, which allowed low-TEA inks to surpass the reactivity of high-TEA inks while increasing print speeds by four-fold. Prints up to 3 cm^3 could now be printed in under 3 minutes. This also improved cell viability from negligible to >85% cell survival in all printed constructs. In addition, cell viability in prints was vastly improved when using high-transmission inks. This IDX concentration improved the print resolution from worse than 1 mm to 70 μm, enabling the printing of complex, biologically relevant, cell-laden structures.

Conclusions
IDX addition was identified as a highly effective strategy to address a key challenge of realizing the promise of bioxolography. Specifically, the use of IDX allows rapid xolographic printing of voluminous living matter at high-viability and high-resolution in seamless, shear stress-free, and cytocompatible manner.

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Subtopic: Advances in Light-Induced 3D printing and Bioprinting and Photo-Crosslinkable Inks: Innovations and Challenges]
Type: Oral presentation

TERMIS25_310 - Bio-Xolography: fast and high resolution volumetric 3D bioprinting of living matter

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Introduction/Objectives
Volumetric bioprinting (VBP) technologies have shown great potential for printing centimeter-scale engineered tissues, with intricate architectures, in minutes [1]. However, VBP still faces limitations with scalability, resolution, and multi-materials printing. Recently, a next-generation volumetric 3D printing, Xolography, was introduced, which works by intersecting UV and visible-light beams in a dual-color photo-initiator (DCPI) print bath. This technique achieves exceptional volume generation rates (55 $mm^3.s^{-1}$) and resolution (<10 μm), while improving resin efficiency and scalability [2]. However, its applications and associated processes have remained limited to the printing of plastics. In this work, we pioneered the bio-xolographic printing of cell-laden hydrogels to enable rapid high-resolution bioprinting of living matter.

Methods
Gelatin methacryloyl (GelMA) was blended with photoreactive compounds to prepare different photopolymerizable formulations. Their reactivity was analyzed via dual-color photorheology and xolography to optimize concentrations, printing speed, and light irradiance for high-resolution printing. Cell-free hydrogels were printed in intricate shapes, and both printing fidelity and resolution were evaluated. To enable 3D-bioprinting, the cytotoxicity of DCPI, co-initiator, and additives was assessed to formulate bioinks that are non-cytotoxic and cell-conducive. Furthermore, cell-laden hydrogels (e.g., with hMSCs, chondrocytes, and cardiomyocytes) were 3D-printed, and cell viability and function were determined. Moreover, complex and biomimetic 3D shapes of living tissues were printed to demonstrate the technique's potential.

Results
Additives in GelMA formulations were essential for xolographic hydrogel printing, enhancing crosslinking rates to address DCPI reactivity limits. We achieved centimeter-scale structures with <25 μm resolution and perfusable architectures within 3 minutes, along with multi-material and spatial patterning capabilities. DCPI concentrations supported >98% cell viability; however, the co-initiator, triethanolamine, showed concentration-dependent cytotoxicity, setting an upper concentration limit (<2.5 wt%). Optimized formulations enabled cell-laden hydrogels with high resolution (<80 μm), >80% viability, and maintained functionality, underscoring bio-xolography's potential for rapid, large-scale tissue biofabrication.

Conclusions
This study demonstrates xolography's suitability for 3D-bioprinting complex, centimeter-scale living matter with high speed and resolution, while preserving cell viability. Bio-xolography emerges as a next-generation technology for creating multi-scale engineered tissues rapidly, enabling the bioprinting and patterning of large, intricate tissue architectures within practical timeframes.
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Acknowledgments: J.L. acknowledges financial support from University of Twente (KI grant) and EIC (ELM project 210803707: BioRobot-MiniHeart). A.W acknowledges financial support from the Dutch Research Council (NWO-XS grant 16998).

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Type: Oral presentation

TERMIS25_419 - Engineering 3D Intestinal Models using DLP Bioprinting: From Crypt-Villus Structures to Dynamic Gut-on-Chip Platforms

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Introduction/Objectives
The small intestine's intricate architecture, characterized by finger-like villi structures, is essential for its role in nutrient absorption and barrier function. Despite its significance, *in vitro* models often fail to capture the three-dimensional (3D) complexity and multicellular organization of the intestinal mucosa, limiting their physiological relevance. In this work, we present a comprehensive approach to engineering 3D intestinal models that integrate epithelial and stromal compartments, using an advanced light-based bioprinting approach.

Methods and Results
First, we customized a digital light projection (DLP)-based bioprinting system and a low macromer content bioink (< 10% w/v), composed by a mixture of gelatin methacryloil (GelMA) and poly(ethylene glycol) diacrylate (PEGDA), enabling the fabrication of 3D crypt-villus structures that mimic the native intestinal architecture by means of visible light photopolymerization (1). This robust method achieved high spatial resolution and throughput, with excellent cell viability (> 90%, 7 days post encapsulation) when fibroblasts are embedded in, laying the groundwork for reproducible models of the gut that include the stromal compartment. We demonstrated the physiological relevance of these constructs by incorporating epithelial cells, including enterocytes and goblet cells, onto the fibroblast-laden 3D printed structures. This multicellular system revealed that both the presence of fibroblasts and the 3D villus-like formations enhanced epithelial barrier formation and function, extracellular matrix remodeling, and drug transporter activity compared to 2D or flat 3D models (2). Notably, P-glycoprotein (P-gp) efflux activity was significantly reduced in the 3D bioprinted model, while the expression of other drug transporters better reflected *in vivo* profiles, highlighting the 3D architecture's critical role in improving drug absorption studies.

Building on these advancements, we developed a hydrogel-based microfluidic gut-on-chip (GOC) platform, that uniquely mimics the 3D intestinal villi architecture and mechanical cues incorporating physiological flow conditions and real-time impedance measurements for intestinal barrier monitoring (3). The system successfully supported the co-culture of fibroblasts and epithelial cells, maintaining the barrier integrity and function under dynamic conditions. By recapitulating both the physical and biochemical microenvironment of the gut, this hydrogel-based platform offers a significant step forward in developing physiologically relevant organ-on-chip models.

Conclusions
Collectively, these studies demonstrate a versatile and scalable approach to engineering the intestinal mucosa's complexity, bridging the gap between *in vitro* models and *in vivo* physiology; holding significant promise for applications in drug screening, disease modeling, and personalized medicine.
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Type: Oral presentation

TERMIS25_1317 - Discovering the Frontiers of Nanoscale Processing via Two-Photon Polymerization: Pioneering Functional 3D Models for an Advanced Myocardium Platform

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Introduction/Objectives
Cardiac tissue models are critical for understanding the molecular fundamentals of cardiac physiology and for advancing regenerative therapies and drug testing. An ideal myocardium-on-chip model replicates the heart's middle muscular layer with a 3D microphysiological environment that promotes cardiomyocyte proliferation, polarization, differentiation, and synchronized beating. This study explores the application of two-photon polymerization (2PP) to fabricate nanostructured, three-dimensional (3D) myocardium-on-chip platforms. These platforms aim to recreate the anisotropic structure and functionality of the myocardium, providing a cutting-edge environment for cardiomyocyte alignment and functionality.

Methods
A 2PP-based fabrication approach was employed to construct nanostructured scaffolds with precise topography. Finite Element simulations optimized scaffold design, focusing on ridge geometry and nanotopography for improved cell alignment. The scaffolds were integrated into a microfluidic device containing a GelMA-based extracellular matrix. r-HL-1 cardiomyocytes transfected with GCaMP6 calcium indicators were cultured on the platform. Immunostaining and FFT analysis assessed cell alignment and activity, while integrated gold electrodes and an impedance analyzer facilitated electrophysiological monitoring. Measurements included ECG recordings and impedance spectroscopy to capture detailed insights into cellular activity.

Results
The 2PP-fabricated scaffolds exhibited excellent fidelity to the simulated designs, with a ñpw deflection error. The scaffolds' mechanical properties were optimized, achieving Young's modulus of between 47 and 200 MPa, suitable for cardiomyocyte attachment and alignment. The nanostructured surface induced significant topographical cues, enhancing cell alignment and functionality. Real-time calcium imaging and electrophysiological monitoring revealed synchronized activity, with the 3D myocardial culture achieving a ∼90% viability rate. ECG recordings showed clear correlations with calcium activity, while impedance spectroscopy provided detailed insights into the electrical properties of the myocardial culture. Reduced calcium activity in 3D cultures compared to 2D controls highlighted the physiological relevance of the platform, which indeed needs to be further optimized.

Conclusions
2PP has been demonstrated as a powerful tool for engineering advanced myocardium-on-chip platforms. The nanostructured scaffolds recreate the anisotropic environment of cardiac tissue, enhancing cardiomyocyte functionality and alignment. These findings represent a step forward in the development of in vitro cardiac models for regenerative medicine and pharmacological testing. Future work will focus on optimizing bio/non-bio interactions and integrating additional functionalities through advanced 3D printing techniques.

Acknowledgments:
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Subtopic: Advances in Light-Induced 3D printing and Bioprinting and Photo-Crosslinkable Inks: Innovations and Challenges]
Type: Oral presentation

TERMIS25_1321 - High-Resolution 3D-Printed Scaffolds for Modeling Angiogenesis: Advancing Tissue Engineering and Drug Discovery

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Introduction/Objectives
Angiogenesis, the formation of new blood vessels from existing vasculature, is essential for processes such as tissue repair, cancer progression, and wound healing. Despite its importance, replicating angiogenesis in vitro is challenging due to the complex and dynamic interactions among cellular and molecular components in three-dimensional environments.

Recent advancements in high-resolution 3D printing have revolutionized tissue engineering by enabling the fabrication of intricate, biomimetic structures, allowing for dynamic, three-dimensional models that capture the complexity of native tissues more effectively than traditional 2D systems. These innovations provide an unprecedented opportunity to create tailored environments for studying angiogenesis in controlled settings.

The goal of this study is to leverage high-resolution 3D printing to fabricate customizable in vitro models of angiogenesis. By integrating biomimetic scaffold designs, advanced biomaterials, and pro-angiogenic factors, the study aims to elucidate the effects of scaffold geometry, material properties, and microenvironmental conditions on vascular network formation.

Methods
The scaffolds were fabricated using two-photon polymerization (2PP), a high-resolution 3D printing technique, allowing for the creation of precise, intricate structures that mimic the three-dimensional architecture required for angiogenesis studies. This approach allowed for precise manipulation of scaffold geometries, facilitating the study of vascular growth within a three-dimensional context. The printing process enabled fine control over scaffold features such as pore size and interconnectivity, which are critical for supporting vascular growth. These scaffolds were designed to promote cell attachment, migration, and proliferation, essential for modeling angiogenic processes in vitro. Several inks with different mechanical properties have been used, ranging from softer inks, such as gelatin-based inks, to stiffer inks such as the commercial DEGRAD INX N100 or inks from Nanoscribe.

Results
Preliminary results demonstrated that the 3D-printed scaffolds provided a robust platform for angiogenesis studies. The scaffolds exhibited high structural fidelity and provided an ideal environment for guiding vascular growth. Variations in scaffold geometry and biomaterial composition significantly influenced angiogenic processes, underscoring the importance of material selection and three-dimensional design in modeling vascular formation.

Conclusions
This study highlights the transformative potential of high-resolution 3D printing for developing advanced in vitro models of angiogenesis. The ability to create customizable, biomimetic scaffolds provides a powerful platform for studying vascular processes in a physiologically relevant context. These findings underscore the importance of scaffold

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design and material properties in guiding vascular formation, advancing both tissue engineering and drug discovery.

Acknowledgments
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Topic: Submit to SYMPOSIUM
Subtopic: Advances in Ocular Tissue Engineering
Type: Oral presentation

TERMIS25_564 - Blue light crosslinkable gelatin-based hydrogels for corneal tissue engineering and bioprinting

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Introduction/Objectives
Corneal damage is among the leading causes of blindness worldwide. The current treatment of choice is corneal transplantation. To address the global donor shortage, corneal tissue engineering aims at generating an artificial cornea to replace the damaged tissue. One approach is to create cellular constructs that can either be implanted or printed in the patient's eye. For this purpose, we examine a hydrogel formulation based on gelatin methacryloyl and using riboflavin and arginine as photoinitiating system. To examine the suitability of the formulation for corneal tissue engineering, the crosslinking process, gel properties and behavior of encapsulated human corneal keratocytes (HCK) was characterized.

Methods
The crosslinking procedure with blue light, and the gel properties were examined by oscillatory rheology. Furthermore, cytotoxicity and phototoxicity were assessed by metabolic assays using immortalized human corneal keratocytes (HCKi). The behavior of encapsulated cells was studied by actin staining as well as immunostaining ten days post-encapsulation. Furthermore, the viability of encapsulated cells was assessed at different time points post encapsulation. In addition, the freeform reversible embedding of suspended hydrogels (FRESH) method was used for bioprinting.

Results
Using riboflavin and arginine as the photoinitiating system, hydrogels can be prepared via blue light irradiation. The gel point can be adjusted between 42 - 300 s by varying the arginine concentration or polymer content. Depending on the arginine concentration, irradiation time, and irradiation intensity, the storage moduli of the hydrogels can be adjusted between 2.5 – 17 kPa. The hydrogel formulation as well as irradiation with blue light are non-cytotoxic. Encapsulation of HCK was optimized by varying the arginine concentration and the irradiation time to enable cell spreading and phenotype conservation. A high viability (above 85 %) was preserved for ten days post-encapsulation. The hydrogel formulation is printable via the FRESH method and encapsulated cells display a high viability ten days post-printing as well as a high degree of cell spreading.

Conclusions
Riboflavin and arginine are a suitable photoinitiation system to prepare Gel-MA-based hydrogels by visible light crosslinking, which is less harmful to cells than conventional UV-light induced crosslinking. As both compounds are biobased, this approach might be also a sustainable alternative to conventionally used synthetic photoinitiators. The formulation is suitable for HCK encapsulation and 3D culture as well as bioprinting and thus paves the way for advanced corneal tissue engineering.

Topic: Submit to SYMPOSIUM
Subtopic: Advances in Ocular Tissue Engineering
Type: Oral presentation

TERMIS25_897 - The influence of the shear stress on a corneal epithelial cells and keratocytes 3D coculture in custom made bioreactor system

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Introduction/Objectives
Mechanical stimulation plays an important role in regulating cell behavior. While several studies have examined how corneal epithelial cells respond to mechanical stimuli such as fluidic shear stress, little is known about how this response affects keratocytes in the corneal stroma. For this reason, the purpose of this study was to design a fluidic system to determine the effect of shear stress on the behavior of corneal epithelial cells and keratocytes in an in-vitro 3D corneal hydrogel model.

Methods
The shape of a PDMS chamber has been optimised through an iterative process until the requirements of isolation, sterility maintenance, and biocompatibility were satisfied.

The custom-made chamber contains 4 wells to host 4 10µl-hydrogels loaded with keratocytes, above each of which a 20µm-membrane seeded with epithelial cells is placed. The hydrogel is made of collagen type I (3.5ml/ml) and 10mM PEG-SG (polyethylene-glycol-succinimidyl glutarate).

The membrane is made of electrospun PCL (polycaprolactone) coated with PDA (polydopamine). After the seeding the chamber is connected to a pumping system to provide a fluid flow over the cells co-culture at different rates.

Results
The bioreactor was successfully assembled and tested for hydraulic sealing and sterility maintenance. The shear stress applied to the surface of the hydrogels was calculated using a simple fluid mechanics model and validated using computational fluid dynamics software.

Preliminary studies using collagen hydrogels showed a high degree of contraction, so PEG was introduced to the hydrogels to assist in stabilizing their dimensions. YAP (Yes-Associated-Protein) is known to be a mechanosensitive protein.

A protocol for the set-up of the co-culture of keratocytes and epithelial cells has been optimized.

Epithelial cells showed a higher nuclear YAP expression after a 24h-stimulation at 1 Pa, confirming that the stimulation occurred. Moreover L&D was performed on keratocytes. High viability was found at 1 and 3 days. These results validated the bioreactor function.

On going studies are comparing the gene expression of epithelial cells markers (ZO-1, CTGF, CK3 and CK12) and keratocytes markers (KERA, LUMI, ALDH1A1, ALDH3A1, COL1, and COL5) after 1 and 3 days of stimulation, under three different shear stress conditions: 0 Pa, 0.25 Pa, and 1 Pa.

Conclusions
The bioreactor has been developed and successfully validated to apply shear stress to corneal hydrogel models. Short term studies, at day 1 and 3, provides a first result on the behavior of cornea cells in response to different magnitudes of shear stress. Further implementations, such as microfluidic system, will allow long term studies at day 7 and 14 to understand possible changes in ECM (extracellular matrix) composition and arrangement.

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Topic: Submit to SYMPOSIUM
Subtopic: Advances in Ocular Tissue Engineering
Type: Oral presentation

TERMIS25_912 - Evaluation of cornea cells' response to hydrogels with tuneable viscoelastic properties

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Introduction/Objectives Cornea blindness can be caused by several factors, including damages induced by a number of injuries and diseases.[1,2] Cornea transplantation constitutes the gold standard treatment, but unfortunately the lack of donors severely impacts the number of patients that can be treated.[3] Artificial scaffolds are a promising alternative, but even though many types of materials and techniques have been investigated, most scaffolds still fail to replicate important features of the native cornea. Time-dependent mechanical properties are a fundamental aspect of the extracellular matrix (ECM) and although their role is less clear compared to other material properties (i.e. stiffness, substrate morphology) it has been demonstrated that they also play an important part in regulating cell behaviour.[4] Therefore, it is crucial to better understand how these type of mechanical properties can affect corneal cells and how by modulating these properties we can achieve better results in engineering scaffolds for corneal regeneration purposes.

The aim of this project is to develop hydrogels that possess variable viscoelastic properties and assess how changes in these properties affect cornea stromal and epithelial cells.

Methods To this end alginate with variable molecular weight (MW), which was modulated by autoclaving, was used as the base material. The different alginates were then mixed with Collagen type I and PEG Succinimidyl Glutarate to produce 3 types of hydrogels, which were then tested for mechanical properties and cytocompatibility. The effects on cells were evaluated through histology, RT-qPCR and immunofluorescence.

Results Gels showed very close values of elastic modulus, but large differences in viscoelastic properties (i.e. stress-relaxation). We demonstrated that these properties can be modulated by varying the concentration of the crosslinker $CaCl_2$, which also affected gels' transparency. Moreover we showed that hydrogel stability in cell culture conditions is correlated to the number of autoclaving cycles (therefore to the MW). Hydrogels proved to be non-toxic to cornea stromal cells, furthermore, remodelling of the gels was observed over the course of 2 weeks which lead to a substantial increase in transparency.

Conclusions We showed a simple system to modulate the mechanical and viscoelastic properties of alginate-collagen hydrogels, furthermore, viscoelastic properties are shown to affect cornea stromal cells behaviour.

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Topic: Biomaterials
Subtopic: Biomimetic, multiphasic, or smart models
Type: Oral presentation

TERMIS25_1141 - Comparative rheological analysis of vitreous humor from sheep, cow, and camel as potential vitreous substitutes

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Introduction/Objectives:
Vitrectomy has become a common procedure for treating several retinal diseases. Since the vitreous humor (VH) cannot regenerate, substitute materials must be filled in the cavity. Slaughterhouse waste, particularly the VH, a clear, gel-like material that fills the gap between the eye's lens and retina, has not been explored much as a vitreous substitute. Native VH possesses few cells and is rich in collagen, hyaluronic acid, and glycosaminoglycans, making it a potential hydrogel material that can be repurposed for vitreous reconstitution/substitution. The challenge lies in replicating its unique rheological properties and biocompatibility to restore ocular function effectively. The objective of this study was to evaluate the rheological properties of the VH obtained from various species, namely sheep, cow, and camel, for its potential use as a vitreal substitute, with a focus on its biocompatibility and structural similarity to the native gel as anticipated benefits.

Methods
This study examines the impact of storage conditions on the rheological properties of VH. Three primary storage conditions were evaluated: freshly processed, short-term storage at -20°C for 24 hours, and long-term storage (>3 months) at -80°C. Rheological analysis included viscosity measurements and amplitude sweep tests to determine the storage modulus (G') and loss modulus (G"). Statistical significance was assessed using ANOVA and t-tests, with p-values < 0.05 indicating significant differences.

Results
The results revealed that sheep samples exhibited higher viscosity and storage modulus compared to cow and camel samples. The average viscosity for sheep samples was 3944.75 ± 1505.8 mPa·s, compared to 2375.75 ± 1194.7 mPa·s for cow and 424.6 ± 119.7 mPa·s for camel samples. In amplitude sweep tests, the storage modulus in the viscoelastic region ranged from 1.9 Pa to 7.0 Pa for sheep, 0.15 Pa to 1.9 Pa for cow, and 0.9 Pa to 3.8 Pa for camel. Statistical analysis revealed that the viscosity of sheep samples was significantly higher than that of the cow and camel samples. Moreover, stored samples demonstrated higher viscosity compared to freshly processed samples.

Conclusions
These preliminary findings indicate that hydrogels derived from sheep vitreous humor may serve as a promising vitreous substitute. However, further detailed physicochemical and biological analyses are required. Additionally, modifications and functionalization, such as optimizing hydrogel crosslinking or incorporating bioactive molecules, could enhance their properties for clinical applications.

Topic: Tissue-Specific Focus
Subtopic: Tendons & ligaments
Type: Oral presentation

TERMIS25_314 - Cellular and molecular landscapes of human tendon development and ageing through comparative histology and single-cell transcriptomics

Alina Kurjan[1], Jolet Mimpen[1], Lorenzo Ramos-Mucci[1], Carla Cohen[1], Cenk Aksu[1], Christopher Buckley[2], Adam Cribbs[1], Mathew Baldwin[1], Sarah Snelling[1]

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Introduction/Objectives
Tendon diseases pose a global health challenge, affecting individuals of all ages and often necessitating surgical interventions with high failure rates. Although tendons can regenerate without scarring during development, this capacity diminishes with age, increasing susceptibility to injuries and impairing repair. This study investigates mechanisms of human tendon regeneration and age-associated decline through comparative histological and single-cell transcriptomic analyses of developing and aged healthy or ruptured tendons.

Methods
Single-nucleus (*N*=15 foetal, 9 donors, 12-20 pcw; *N*=26 adult, 12 donors, 25-84 years) and spatial RNA-sequencing (*N*=3 foetal, 1 donor, 20 pcw) were performed on Achilles and quadriceps tendons. Tissues were stained with haematoxylin & eosin, Masson's trichrome, alcian blue, and picrosirius red. Additionally, single-cell (*N*=25; 11 donors, 5.1-9.3 pcw) and spatial (*N*=8; 2 donors, 8 pcw) RNA-sequencing data from embryonic limbs were sourced from Zhang *et al.* (2023). A random forest classifier trained on spatial tendon regions was applied to single-cell data to identify embryonic tendon cells (6 donors, 6.5-9.3 pcw).

Results
Single-nucleus and spatial RNA-sequencing analyses identified 13 distinct cell types within 12-20 pcw foetal human Achilles and quadriceps tendons, including immune, endothelial, and neural populations, alongside myocytes, chondrocytes, and 5 unique fibroblast subsets. The predominant fibroblast population within tendon fascicles showed high expression of *ABI3BP*, *COL1A1*, *KERA*, and tendon stem/progenitor cell (TSPC) markers *SCX*, *MKX*, *TNMD*, *ENG*, *THY1*, *CD44*, and *NES*, indicating a role in extracellular matrix (ECM) expansion. Fibroblasts in tendon loose connective tissue layers expressed markers like *COL3A1*, *VCAN*, *COL6A6*, *LUM*, and sheath TSPC markers *TPPP3, PDGFRA*, with an overall profile aligning with functions in tendon morphogenesis, repair, and integration with surrounding tissues. Additional fibroblast populations, including myotendinous junction (MTJ)-localised *COL22A1*-expressing cells, were identified.

Developmental trajectory analysis of embryonic populations in 6.5–9.3 pcw tendons identified seven precursor states from a common progenitor that differentiated into three anti-correlated lineages, contributing to the foetal SCX^+ fascicular fibroblasts, SCX^- loose connective tissue fibroblasts, and chondrocytes in foetal tendons.

Comparative analyses between foetal and adult tendons revealed major differences in cellular composition, morphology, and ECM organisation. Adult tendons contained adipocytes, an expanded immune cell repertoire, and transcriptionally distinct, maintenance-focused fibroblasts lacking TSPC markers. In ruptured aged tendons, a partial reactivation of developmental repair programs was observed, though limited in scope and efficacy.

Conclusions
These findings elucidate the cellular mechanisms of tendon development and ageing, identifying key cell types and their functions. Regenerative tendon development is driven by distinct fibroblast populations, including TSPC marker-expressing fascicular and sheath fibroblasts, which contribute to ECM expansion and structural maturation. In contrast, adult tendons possess transcriptionally distinct, maintenance-focused fibroblast populations lacking TSPC markers, leading to limited repair capacity in aged or ruptured tendons.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_357 - A Hydrogel Scaffold for Effective mRNA delivery in Tendon Repair

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Introduction/Objectives
Tendon repair remains a significant clinical challenge, as tendons have inherently poor regenerative capacity, often leading to scarring and high re-tear rates. This underscores the urgent need for therapies that can regenerate the healthy, functional tendon tissue. In recent years, mRNA has garnered considerable interest in disease intervention and tissue regeneration. However, the successful translation of mRNA therapeutics for tendon regeneration faces unique challenges. Specifically, tendon undergoes a slow and complex healing process that can take months or even years, necessitating the development of mRNA delivery systems capable of providing sustained bioactivity. While efforts on mRNA chemical modification and lipid nanoparticle (LNP) packaging have been critical, an equally important aspect is the establishment of a biomaterial-based localized mRNA delivery system, such as hydrogels, to achieve spatiotemporal control over the release kinetics and bioactivity of the encapsulated mRNA. However, the optimal duration for transgene expressions to regulate the best tenogenic response are not yet fully understood. Investigating how the processes of vector release from scaffold systems and transgene expression therefore represents the net frontier to enhance tendon repair *in vivo*. In this study, we have developed a TenoGel scaffold that enables controlled and sustained release of mRNA-LNP, and also allows us to study how this release affects tendon repair.

Methods
1) Synthesis and characterization of mRNA-LNP: mRNA-LNP will be synthesized as previously described[2] and the hydrodynamic diameter and surface charge of LNP will be characterized by dynamic light scattering (DLS) and zeta-potential. 2) Development and characterization of the TenoGel: To characterize the morphology, mechanical property and ROS cleavage property of the hydrogel, compression tester, scanning electronic microscope (SEM) and total antioxidant test were performed. To test the biocompatibility of the hydrogel, hASCs were incorporated and co-cultured in growth medium for 48 hours before assessing using Live/Dead assay (Invitrogen). To assess the releasing kinetics of mRNA, the TenoGel loaded with cy5-labeled mRNA-LNP will be incubated in PBS under 37 ^{o}C and the supernatant will be measured by fluorescence plate reader to measure released mRNA at predetermined intervals. 3) *In vitro* sustained transfection study: Gluc mRNA-LNP loaded TenoGels were incubated in growth medium, and the supernatant was collected every 2 days. hASCs were seeded on the well plate at a density of 10,000 cell/cm^2 and cultured with the supernatant for 24 hours before analyzed using a luciferase assay.

Results
The synthesized mRNA-LNPs were well-monodispersed, with an approximate size of 90 nm and a neutral surface charge. The TenoGel exhibited a fibrous structure with improved modulus and ROS cleavage properties. Different TenoGel formulations effectively modulated release profiles and transfection efficiency/duration of mRNA-LNP, for up to 10 days *in vitro*.

Conclusions
In summary, this study investigated an mRNA delivery system, packed in LNP and TenoGel to achieve sustained release for tendon regeneration. Future studies will focus on evaluating *in vivo* transfection efficiency and its effects on promoting tendon healing.

Topic: Tissue-Specific Focus
Subtopic: Tendons & ligaments
Type: Oral presentation

TERMIS25_606 - Development of tissue-engineered substitutes for tendon total ruptures

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Objectives
Tendons are elastic and resilient connective tissues that enable force transmission between muscles and bones. However, total ruptures are common and challenging to treat effectively. Tissue engineering strategies appear as a promising solution, but achieving fully functional tendon tissue remains elusive. This is mainly due to the lack of resilient support materials that promote functional tissue formation while withstanding physiological loading conditions.

This study aims to create 3D-bioprinted tissue-engineered substitutes based on highly elastic polyesterurethanes combined with heparin, that closely mimic the extracellular matrix (ECM) composition and mechanical properties of native tendon.

Methods
Fabrication of cell-laden scaffolds. Two methacrylated polyesterurethanes were synthetized: 2-hydroxyethyl methacrylate (HEMA) and polyethylene glycol methacrylate (PEGMA). Biocompatibility was assessed in 2D using bone marrow-derived mouse mesenchymal stem cells (MoMSCs) via lactate dehydrogenase (LDH) release and live/dead assays. MoMSCs were encapsulated within methacrylated bioinks with varying heparin concentrations. Cell-laden scaffolds were fabricated via extrusion-based 3D bioprinting. Cell viability and proliferation were evaluated by live/dead staining, LDH release and DNA quantification. **Mechanical characterization.** Various scaffolds with different printing patterns were fabricated and their mechanical properties tested under tension, analyzing creep and resilience. **Tenogenic differentiation.** A tenogenesis protocol was established in 2D to drive MoMSCs towards tenocytes, and the stability of this phenotype was evaluated over 28 days of culture by qPCR and immunofluorescence (IF). 3D-bioprinted constructs with and without heparin were cultured in tenogenic media for 28 days. Cell differentiation potential was analyzed by means of qPCR and IF. ECM deposition and tissue formation were evaluated by GAG quantification and scanning electron microscopy (SEM).

Results
Cell-laden HEMA and PEGMA scaffolds were 3D-bioprinted. MoMSCs in HEMA scaffolds exhibited higher viability and proliferation as compared to cells in PEGMA. Optimal scaffold patterns were selected to recreate the mechanical properties of native tendon. The tenogenesis protocol effectively induced a tendon-like phenotype in MoMSCs, upregulating specific genes (scleraxis, tenomodulin and tenascin-C) and enhancing the synthesis of tendon-related proteins such as collagen I, III and tenascin-C. 3D bioprinted scaffolds cultured in tenogenic media demonstrated effective differentiation of encapsulated MoMSCs toward tenocytes, showing the deposition of tendon-characteristic proteins such as collagen I and tenascin-C. The inclusion of heparin enhanced the synthesis of matrix assembly proteins and increased the GAG content within the 3D-bioprinted constructs.

Conclusions
Cell-laden scaffolds were successfully fabricated by 3D-bioprinting, supporting cell viability and proliferation within the 3D constructs. The selected design parameters enabled the creation of constructs that recreate the resilient mechanical properties of native tendon tissue. The tenogenic differentiation protocol induced the formation of tendon-like tissue within the 3D-bioprinted constructs and the addition of heparin enhanced tissue formation and organization, showing promising results for the regeneration of tendon total ruptures.

Topic: Submit to SYMPOSIUM
Subtopic: Advances in Tendon and Ligament Regeneration: Integrating Research, Bioengineering, and Clinical Practice.
Type: Oral presentation

TERMIS25_1206 - From Force to Function: Modelling Frozen Shoulder with Mechanical Stimulation

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Objectives: Previous work identified macrophages and fibroblasts are key cell types implicated in frozen shoulder (FS) resolution (Ng et al 2023). This work sought to develop a 3D patient-derived organoid model to recapitulate FS to advance understanding of mechanisms underpinning fibrosis resolution, uncovering treatment targets for fibrotic joint disease.
Methods: Patient-derived organoids (PDOs) were generated from three cell types; fibroblasts, macrophages and HUVECs, suspended in Matrigel™. Primary fibroblasts were isolated from FS or comparator tissue explant cultures (passage 2-3). Monocytes were isolated from pooled donor blood and differentiated into macrophages (M0) in 2D. HUVECs were routinely cultured and harvested at P4-6. A Matrigel cell suspension was plated in droplets on low adhesion plates. Subsequently, PDOs formed and were cultured in suspension. To explore macrophage-fibroblast cross-talk, we examined LPS, DEX, PROS1 and ADAM17's influence on macrophage's MerTK expression in 2D, followed by FACS. To provide mechanical stimulus, organoids comprising a fibroblast cell-line were preliminarily stimulated using ultrasound. These were fixed, embedded, sectioned and stained stained for mechanosensitive markers including TRPV4, IL-R1, Vinculin and Piezo1, and imaged on a slidescanner. Confocal images of PDOs were taken after antibody incubation against Ki67, CD146, and nuclear stain POPO-1. Whole PDOs were stained with DKK3, CD31 and POPO-1 to view fibroblasts, HUVECs and nuclei respectively using confocal z-stack imaging.
Results: DEX stimulation induced the highest MerTK expression, followed by PROS1 and unstimulated cells, while LPS reduced it. ADAM17 inhibitor reduced MerTK shedding. Preliminary results showed mechanical stimulation leads to an increase in cell viability as well as mechanosensitive markers IL-R1 and Vinculin, consistent with FS tissue. Confocal imaging of stained PDO sections revealed a distinct lining and sub-lining region resembling synovial tissue. FS organoids displayed a thickened lining layer with elevated CD146 staining and Ki67 expression, indicating increased fibroblast activation and proliferation. Z-stack imaging revealed evenly distributed HUVECs forming tube-like networks in PDOs. 3D projections showed a more defined HUVEC network in FS PDOs, reflecting the disease's heightened vascularization.
Conclusions: We successfully identified and refined two highly desireable features soon to be included in our PDO model; specific approaches to drive macrophages toward either a pro-resolving or pro-inflammatory phenotype in 2D, and ultrasound mediated mechanical stimulation of fibroblast-laden organoids. PDOs currently recapitulate histological features of FS patient tissue such as a thickened capsule lining and increased vascularity. Fibroblasts, macrophages, and HUVECs self-organized. Notably, HUVECs formed tubes on Matrigel in 2D but not when embedded by themselves, suggesting that tube formation in PDO depends on crosstalk between co-cultured cells. In the absence of representative animal models, this PDO model offers a novel platform for studying disease mechanisms and testing therapies.

Rerefences
1. Ng MTH, Borst R, Gacaferi H, et al. Primed to resolve: A single cell atlas of the shoulder capsule reveals a cellular basis for resolving inflammatory fibrosis. Published online 2023:1-50.

Topic: Cancer
Subtopic: Cancer models
Type: Oral presentation

TERMIS25_762 - Development of Three-Dimensional Organotypic Bone Metastasis Models

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Introduction/Objectives
Metastatic spread of solid tumors, particularly from breast and prostate cancers, has a devastating impact on patients' quality of life and

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survival. Bone is a common site of metastasis, affecting over 1.5 million cancer patients worldwide, with a healthcare expense of US $13 billion. The interactions within the bone microenvironment and the dysregulated extracellular matrix (ECM) complicate the development of effective treatments. Current therapies are mainly palliative and focus on life quality support. The development of a human bone-like microtissue and co-culture with cancer cells requires 8–12 weeks for cells to grow and differentiate into bone-like tissue. Despite the extended culture period, minimal ECM deposition occurs, which may contribute to the high failure rate of anti-cancer agents in clinical trials. Since ECM plays a key role in cancer progression, optimal ECM deposition is critical for accurate modeling. In traditional cell culture, the enzymatic conversion of procollagen to collagen occurs slowly, leading to delayed ECM deposition. Macromolecular crowding (MMC) is a biophysical process that accelerates ECM deposition by reducing molecular diffusion and enhancing protein interactions. The introduction of MMC into in vitro cultures could mimic in vivo conditions more closely, potentially bridging the gap between promising research findings and clinical success. MMC has been used to improve in vitro scar models and decellularized matrices, but its potential in bone metastasis models remains unexplored. This study aims to generate a human bone-like microtissue using hBM-MSCs and co-culture it with metastatic breast cancer cell lines for drug testing.

Methods
Spatially defined patterns will be created by sealing borosilicate cloning cylinders on a plate using sterile silicone grease. hBM-MSCs will be seeded in the outer ring and preconditioned with MMC for 5, 8, or 11 days, followed by 21 days in osteogenic medium. In scaffold-based experiments, CaP-coated mPCL scaffolds will be seeded with hBM-MSCs, preconditioned with MMC, and cultured in osteogenic medium for 21 days. Afterward, cancer cells (MDA-MB-231 and MCF-7) will be seeded in the inner areas or on constructs and co-cultured to observe migration and interactions. Anti-cancer therapeutics, such as bisphosphonates and denosumab, will be tested by optimizing concentrations and evaluating effects on cancer-related genes and apoptosis at selected time points.

Results
We expect that MMC preconditioning of hBM-MSCs will enhance ECM deposition, particularly collagen I, resulting in a bone-like microtissue with increased stiffness and structure. The deposited ECM should closely resemble native bone tissue. We anticipate that upon co-culture with breast cancer cell lines, the degree of cancer cell infiltration will be higher in MMC-preconditioned cultures compared to controls. Furthermore, we expect that treatment with bisphosphonates and denosumab will reduce cancer cell proliferation and increase apoptosis levels.

Conclusions
This study aims to develop a three-dimensional organotypic bone metastasis model using MMC to better replicate native bone tissue. The model could predict cancer cell behavior in response to therapeutic interventions, offering insights into potential treatments for bone metastasis.

Topic: Cancer
Subtopic: Tumor microenvironment
Type: Oral presentation

TERMIS25_789 - Development of a vascularized Tumor-on-Chip platform for dynamic simulation of the tumor microenvironment

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Introduction/Objectives
Advanced in vitro models capable of closely replicating the complex tumor microenvironment (TME) are crucial for following tumor progression in vitro and testing the effectiveness of treatments. Among these, Tumor-on-Chip (ToC) systems stand out for their potential to bridge the gap between traditional 2D and 3D cultures, animal models, and clinics. This study introduces a vascularized alginate-based hydrogel integrated into a custom dual-chamber microfluidic chip, specifically designed to emulate tumor vasculature and physiological dynamics.

Methods
The device development relied on a multidisciplinary approach, combining computer-aided design (CAD), stereolithographic 3D printing, laser cutting techniques, and hot press to create precise geometries. For example, a mask featuring 0.5-mm diameter pins, was 3D printed and enabled the formation of vascular-like channels within the alginate hydrogel. These channels maintained their structural integrity after a crosslinking procedure of the hydrogel (using filter papers and a 3% w/w $CaCl_2$ solution) optimized to ensure biocompatibility and reproducibility. The dual-chamber design of the microfluidic chip was tailored to promote dynamic flow conditions essential for mimicking physiological environments. Each chamber supports distinct functionalities: one for introducing nutrient-rich fluid and simulating blood flow through the vascular network, and the other for confining the tumor-like hydrogel structure. The chambers are interconnected by vascularized hydrogel, which serves as a 3D matrix to support tumor cell growth and interaction. The chip's architecture and transparency also facilitate real-time monitoring and analysis, enhancing its utility in experimental applications.

Results
The results confirmed the mechanical stability of the gel, withstanding perfusion, hot press conditions and ethanol exposure during chip integration, and prolonged handling without deformation or rupture. Moreover, the chip geometry was optimized to ensure proper housing of the gel construct, preventing any leakage during flow and maintaining a continuous interface between the microfluidic system and the scaffold. Extensive validation tests were conducted to evaluate the system's performance under dynamic conditions. Microscopic analysis confirmed the uniformity and stability of the vascular channels over extended flow periods, with no observable deformation or leakage. Fluid dynamics testing using a dye demonstrated controlled flow rates and even distribution within the channels, replicating physiological flow patterns. Additional control tests were performed using gel constructs without pre-formed channels: while the entire gel structure became uniformly colored over time, the pre-defined channels in the system maintained a distinct dye color, further validating the precision of the channel fabrication process.

Conclusions
These findings highlight the system's potential to simulate angiogenesis and TME-specific processes, offering a versatile platform for preclinical research. Unlike static models, this system allows for the application of dynamic stimuli, better mimicking in vivo conditions and enabling more accurate assessments of therapeutic strategies. Furthermore, the modular nature of the dual-chamber chip adapts to a wide range of research applications, including studies on other pathologies involving complex microvascular interactions.

Topic: Cancer
Subtopic: Cancer models
Type: Oral presentation

TERMIS25_854 - Bone metastatic niche: advancing prospective models for investigating prostate cancer and bone microenvironment interactions

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Introduction/Objectives
Prostate cancer (PCa) tends to spread to the bones, causing abnormal deposits of mineralized tissue. To better understand this disease, we aimed to develop an in vitro fully human bone metastatic niche (BMN) model replicating the bone microenvironment in which metastatic castration-resistant prostate cancer (mCRPC) spreads. Our goal is to lay the groundwork for an engineered platform capable of recapitulating the morphology, microenvironmental cues, and key processes involved in PCa bone metastasis.

Methods
Human mesenchymal stromal cells (MSCs) were cultured on hydroxyapatite (HA) scaffolds in osteogenic medium to generate osteoblastic niches, undergoing a 7-day proliferation phase followed by 21 days of osteoblastic differentiation. These osteoblastic niches were sequentially enriched with donor-derived osteoclast progenitors and with PCa cell lines (PC3-mCherry, LNCaP-GFP) to obtain the BMN. The BMNs were characterized using scanning electron microscopy (SEM), immunofluorescence, and immunohistochemistry to confirm key structural and cellular features. PCa cells were sorted from the BMNs, and bulk gene expression analysis of both bone and cancer cell fractions was performed via RT-qPCR.

Results
BMNs exhibited markers of both osteoblast and osteoclast differentiation, demonstrated at the gene level (BGLAP, BMP2, OPN, TRAP) and protein level (BGLAP, IBSP, TRAP). SEM imaging and H&E staining confirmed the deposition of a dense extracellular matrix. Prostate cancer cell lines, LNCaP and PC3, infiltrated the BMNs, displaying differences in morphology and niche penetration capability. Gene expression analysis revealed differential regulation of key osteoblastic and osteoclastic markers within the BMNs, with PC3-laden niches showing significantly reduced TRAP, OPN, and CTSK expression compared to PCa-free controls. Furthermore, PC3-laden BMNs without osteoclasts exhibited increased BMP2 expression and reduced BGLAP levels. Notably, co-culture induced osteo-mimicry in sorted PCa cells, with both LNCaP and PC3 cells expressing TRAP, OPN, and SPARC. PC3 cells also modulated BMP2 expression in a donor-dependent manner.

Conclusions
Our findings demonstrate that the developed model holds significant potential for studying tumor-induced osteogenesis and the regulation of the bone microenvironment in metastatic castration-resistant prostate cancer (mCRPC). Future efforts will focus on enhancing the model by incorporating patient-derived prostate cancer organoid lines, paving the way for a fully personalized platform. This advancement could enable the identification of patient-specific therapeutic targets, contributing to more effective and tailored treatments for individuals with advanced-stage PCa, ultimately improving their quality of life.

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Topic: Cancer
Subtopic: Cancer models
Type: Oral presentation

TERMIS25_1069 - Simplified 3D model for invasion testing: complexified interactions between osteosarcoma cells and bone-mimicking PLGA/20TCP scaffolds

Ewa Walejewska[1], Aleksandra Starosz[2], Marzena Zychowicz[3], Kamil Grubczak[2], Wojciech Swieszkowski[1]

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Introduction
Osteosarcoma (OS), a rare and aggressive primary bone tumor affecting children and adolescents, poses significant challenges in diagnosis and treatment due to its complex microenvironment and rapid progression. Traditional two-dimensional (2D) models fail to replicate the structural and biological complexity of bone and tumor tissue interactions. This study aimed to develop a hybrid 3D osteosarcoma model replicating interactions between healthy and malignant bone tissues. The research focused on three goals: (1) fabricating scaffolds mimicking healthy cancellous bone (BM), (2) developing hydrogels replicating unmineralized bone matrix (osteoid) combined with osteosarcoma spheroids (TM), and (3) integrating these into a hybrid model (BTM) to assess tumor invasion and cell interactions.

Methods
Healthy bone-mimicking scaffolds were fabricated using TCP-enriched (20 wt%) PLGA and PLCL via precise extrusion deposition with a 0/90 fiber orientation and filament shift in the n+2 layer. Scaffolds were characterized for material properties (AFM, SEM, μCT) and degradation (Mw). Scaffolds seeded with hFOB, HUVECs, and hBMSCs in a 1:1:1 tri-culture were evaluated for ALP and CD31 expression via spectrophotometry and immunofluorescence, along with osteocalcin and YAP/TAZ expression. A dual-crosslinked (DC) hydrogel (5% GelMA, G5-DC) was formed via physical (5°C, 1h) and visible light (13s) crosslinking and analyzed for rheological properties, porosity, and biological activity (ALP in hBMSCs). MG63 spheroids (2×10^4 cells) were embedded in G5-DC hydrogels and co-cultured with bone-mimicking scaffolds. The integrated BTM was studied for cell invasion, cytokine production (IL-6, IL-10 (ELISA)), and cytotoxicity (LDH).

Results
PLGA/20TCP scaffolds were chosen for their superior mechanical properties and ability to support bone tissue formation. With a compressive modulus of ~3.70 GPa compared to ~0.64 GPa for PLCL/20TCP, PLGA/20TCP provided the stiffness necessary for osteogenesis. These scaffolds demonstrated enhanced cell adhesion and osteogenic differentiation, shown by increased ALP activity (~120 μM pNP/ng DNA by day 14) and osteocalcin and YAP/TAZ expression alongside robust CD31 expression by day 21. Dual crosslinking strategy produced an osteoid-like stiffness of ~17 kPa. MG63 spheroids were incorporated into G5-DC hydrogels and co-cultured with bone-mimicking scaffolds, forming the integrated BTM. Fluorescence microscopy revealed local cell invasion only into the osteoid-like matrix in the BTM-tumor model (control, spheroids embedded into G5-DC without a seeded scaffold). However, in the full BTM with osteogenically differentiated PLGA/20TCP scaffolds, metastatic spread was inhibited, with IL-6 levels reduced from ~$1.6x10^3$pg/mL (day 3) to ~0.5 $x10^3$ pg/mL (day 32) and IL-10 levels gradually inclining after day 6, a trend in opposite to the BTM-tumor. This suggests that bone-mimicking scaffold plays a crucial role in creating a less permissive environment for tumor progression.

Conclusions
Our findings highlight the utility of BTM as a platform for studying osteosarcoma dynamics and testing therapeutic strategies. We are currently evaluating bone-specific markers to gain deeper insights into the mechanisms underlying the lack of tumor invasion when the bone-mimicking scaffold is introduced.

Acknowledgments
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Topic: Biomaterials
Subtopic: Functionalized, stimuli responsive biomaterials
Type: Oral presentation

TERMIS25_1155 - Selenium and magnesium functionalised scaffolds for dual bone regeneration and anti-cancer therapy

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Introduction/Objectives
Bone related cancer defects are difficult to treat due to disrupted bone formation and bone resorption inhibiting regeneration, and the risk of recurrence from residual cancer cells. Incorporation of bioactive ions into biomaterials has gained remarkable attention for bone regeneration. Magnesium (Mg) is capable of modulating bone resorption and formation to promote bone repair. Selenium (Se), has shown positive results as a cancer therapeutic exhibiting pro-oxidant and apoptosis-inducing effects against cancer cell lines. Nanohydroxyapatite nanoparticles (nHA) are excellent candidates for ion delivery as their crystal structure can accommodate ionic substitution. This project will utilise nHA for the dual delivery of Mg and Se to inhibit bone resorption, augment bone formation and obliterate cancer cells. Specific aims of this project include developing and characterising combined Se and Mg functionalised nHA to augment the therapeutic effects of these ions followed by Se/Mg-nHA particles being incorporated into a collagen-based scaffold to develop a multifunctional bone regenerative and anti-cancerous scaffold for the treatment of bone cancer defects.

Methods
A range of Se, Mg and combined Se/Mg functionalised nHA particles (0 - 50 mM) were developed and characterised. Physiochemical characterisation included Fourier Transform Infrared Spectroscopy (FTIR), Electrophoretic Light Scattering (ELS), and Dynamic Light Scattering (DLS) analysis. RAW-264.7 cells, mesenchymal stem cells (MSCs) and prostate cancer (LNCaP) cells were cultured in 2D with the concentration range of nanoparticles to determine the cytotoxic limits of the ions, using quantitative assays such as Alamar Blue™ and Pico Green™. Collagen-nHA scaffolds loaded with the functionalised nHA nanoparticles were fabricated and biocompatibility of the ion-loaded scaffolds is currently being carried out.

Results
Se, Mg and Se/Mg were successfully incorporated into nHA nanoparticles. FTIR spectra shows characteristic peaks of nHA at 560, 602 and 1019cm-1 with additional peaks observed with incorporation Se, Mg and Se/Mg into nHA. DLS and ELS displayed optimal size and charge of the ion-functionalised nHA. A concentration dependent effect of all three nHA groups was observed in both osteoclast and MSC activity. The highest concentration, 50mM, significantly reduced osteoclast activity in all groups. 1-10mM Mg-nHA significantly increased osteoclast proliferation. However, Se countered this effect in the Se/Mg-nHA group by Day 3, with no increase in proliferation observed between Day 3 and Day 7. MSC proliferation was inhibited at 25-50 mM in all groups. Combination of Se/Mg-nHA significantly upregulated MSC proliferation at lower concentrations (1-10mM) compared to Se or Mg alone.

Conclusions
Based on these results, there is a clear concentration dependent effect of each ion group on both osteoclast and MSC behaviour. 10mM appears to be the optimal concentration for Se, Mg and Se/Mg-nHA, with these nanoparticles displaying a synergistic effect of halting proliferation of pre-osteoclasts while enhancing MSC proliferation at this concentration. Future work involves completing biocompatibility of the ion-loaded scaffolds on bone cells and LNCaP cells to identify the optimal therapeutic concentration. Following this, differentiation and functional activity of osteoclasts and osteoblasts will be assessed in 3D.

Topic: Cancer
Subtopic: Cancer models
Type: Oral presentation

TERMIS25_1233 - GelMA models of human adipose tissue identify deregulated lipid metabolism in the prostate cancer-adipocyte crosstalk in bone and response to antiandrogen therapy both in vitro and in vivo

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Introduction/Objectives
Bone is a common site in advanced prostate cancer (PCa), with survival rates under two years at diagnosis, despite targeted antiandrogen receptor (AR) therapies (bicalutamide, enzalutamide). In the bone tumor niche, increasing evidence reports bone marrow adipocytes as a potential contributor to metastasis, linking hormone therapy and fat metabolism. A key issue in elucidating the role of the microenvironment and assessing drug effectiveness is however hampered by a lack of relevant models. Most *in vitro* models use: 1) cells cultured in 2D (aberrant phenotypes) or; 2) a poorly relevant membrane-basement-extract derived from a mouse tumor (Matrigel) as a 3D cell culture platform. Matrigel contains contaminants and lacks the ability for biophysical manipulation, leading to poor matching of mechanical and biochemical signals of the native microenvironment. Our objective was to use more relevant semisynthetic hydrogels for 3D cell culture of human PCa cells and human adipocytes as more accurate cell culture platforms.

Methods
We used functionalized gelatin- and hyaluronic acid-methacryoyl-based hydrogels (GelMA, HAMA) for human bone-marrow MSCs (BM-MSCs) or human preadipocytes (SGBS) differentiation into adipocytes. We cocultured the adipose microtissues with AR-positive PCa cells (LNCaP, C4-2B) grown in spheroids in separate GelMA hydrogels and assessed gene (RT-qPCR) and protein (ELISA, immunofluorescence) levels and lipids (Nile Red, immunofluorescence) ±enzalutamide (10 μm). Seahorse analysis (Fatty Acid Oxidation (FAO), mitochondrial respiration (OCR), glycolytic function (ECAR)) was done on 2D adipocytes, while adipocyte delipidation was assessed in both 2D/3D ±enzalutamide and ±coculture with cancer spheroids.

Results
GelMA hydrogels supported superior adipogenic differentiation. Upon indirect 3D coculture of mature adipocytes with PCa spheroids, molecular alterations showed increase in leptin gene expression in adipocytes and increase in leptin protein and receptor levels in LNCaP spheroids. FAO, OCR and ECAR were all elevated with Enzalutamide. Delipidation in SGBS was significantly observed only in the 3D model (non-significant in 2D) and in cocultures, and FABP4 was upregulated in PCa spheroids under enzalutamide. The indirect coculture of mature adipocytes with PCa spheroids led to a significant increase of spheroid size ($p<0.0001$), which was mitigated by Enzalutamide.

Conclusions
The ECM-derived hydrogel models presented here address a fundamental problem in the way cancer research is performed, leading to more insightful mechanistic discovery and therapy assessment for the treatment of prostate cancer bone metastasis.

Topic: Submit to SYMPOSIUM
Subtopic: Advancing Bone Cancer Research: Models and Therapies
Type: Oral presentation

TERMIS25_1432 - A Design of Experiments approach to identify key design parameters for topographically-patterned biomaterials in 3D Osteosarcoma Modelling

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Introduction/Objectives
Osteosarcoma (OS) is an aggressive primary malignant tumour, mainly arising in the long bones. Despite advances in drug discovery, patient outcomes have not improved significantly for the past 30 years. To discover new OS therapies and study its progression,

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more physiologically representative models are needed. Three-dimensional (3D) *in vitro* models better recapitulate the complexity of tumour microenvironment compared to 2D models by incorporating critical physical matrix cues. We used a Design-of-Experiments (DOE) approach to fabricate novel topographically-patterned poly(-lactic acid) (PLA) microparticles as substrates for 3D OS cell culture to better mimic key aspects of its extracellular matrix. This strategy removes the time-consuming need for trial-and-error, allowing the design of optimal 3D substrates for modelling osteosarcoma in a dish.

Methods
Microparticles (MPs) of varying surface topographies were fabricated as reported previously. A DOE model of four factors and three levels, employing a central composite design, was used, followed by assessment of the model's robustness. The outputs measured were microparticles size using Dynamic Light Scattering, and dimple size using ImageJ. To assess 3D cell-material interactions, two established osteosarcoma cell lines, MG-63 and U2OS (representing fibroblastic and epithelial phenotypes, respectively), were cultured on different microparticles designs. Viability was assessed with ReadyProbes Cell Viability Imaging Kit, and cell-substrate aggregate morphology was evaluated using Calcein-AM. Automated image analysis was carried out using ImageJ.

Results
Homogenization speed and polymer-to-sacrificial phase ratio were among the dominant influencers on microparticle and dimple sizes. Prediction plots for MPs size and dimple size showed R^2 of 0.93 and 0.87, respectively, with R^2 values for external validation of 0.89 and 0.35, respectively. This may indicate non-linear relationships or higher-order interactions not fully captured by tested parameters. Validation feedback was used to improve prediction accuracy. Cell-MP aggregate sizes were significantly different across the 3 microparticles designs. Both cell lines formed significantly larger aggregates ($p<0.0001$) when cultured on smooth microparticles relative to textured ones, e.g. U2OS cells cultured on smooth MPs displayed mean aggregate size of 166,377 μm^2 relative to 40,063 μm^2 on textured microparticles. Moreover, mean cell-MP aggregate count was notably different ($p=0.069$) in MG63 cultures on smooth microparticles compared to the textured design. This may be attributed to differences in available surface area affecting cell-MP aggregates' formation, with cells having more opportunities to interact with particles, promoting early 'nucleation' before cells combine into larger aggregates. Additionally, U2OS cells showed reduced viability on textured MPs relative to smooth controls, while MG-63 cells showed high viability on all designs.

Conclusions
MPs size can be reliably predicted with our model, with 3 fabrication factors exhibiting statistical significance in predicting design outcomes. Moreover, systematically analysing aggregate formation dynamics and cellular spatial organization demonstrated that 3D-topography significantly influences architecture and viability response of 3D OS models.

Funding
Academy of Medical Sciences [SBF008\1057]

Topic: Tissue-Specific Focus
Subtopic: Wound healing
Type: Oral presentation

TERMIS25_265 - Melt Electro-Written Polycaprolactone scaffolds with Stem Cells for Treatment of Complex Cavity Wounds

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Introduction/Objectives
Patients with complex cavity wounds from severe trauma or chronic conditions present daily to our hospital and are difficult to manage. The wounds involve large-scale damage to skin, muscle, and connective tissues, and are characterized by poor tissue regeneration. Conventional treatments including dressings, negative pressure wound therapy, and grafts often are inefficient in promoting healing. Tissue engineered solutions using stem cells and customized scaffolds may provide a step change in the treatment of these patients. We present findings into scaffold design and cellular response of a tissue engineered construct designed from a clinical perspective and developed according to Good Manufacturing Practice (GMP) principles.

Methods
Melt-Electrowriting (MEW) of 30μm diameter medical grade polycaprolactone (PCL) in box-like structures of 500μm thickness were optimised for cell adherence by plasma cleaning and sterilisation by 25kGy gamma irradiation. Adult mesenchymal stem cells (MSC) were seeded onto the PCL scaffolds (with or without a coating of medical grade collagen) either as single cells or spheroids. MSC expression of 84 genes relevant to wound healing was determined by PCR arrays. We also trialled a separation layer of 500μm MEW between single and spheroid cell layers to elucidate the effect of paracrine signalling. Results from these trials guided preclinical testing in vivo. Three groups of C57BL/6J mice (n=5/group) received subcutaneous implants (scaffold only, scaffold with cells, and scaffold with collagen and cells). Explanted tissue was assessed histologically after 7 and 14 days to determine responses in a complex biological environment.

Results
Comparison of MSC gene expression on MEW scaffolds in vitro revealed significant differences between single cells (with or without collagen), spheroids, and with a spacer layer. Quantitative differences up to 10-fold were observed for spheroids including upregulation of immunomodulation, chemokines and growth factors including CTG, FGF2, HBEGF, IL6, whilst angiogenesis factors ANGPT1 and VEGFA were reduced in spheroids. Expression of the stemness gene OCT4 was higher in spheroids. With the spacer layer between single and spheroid cell scaffolds, paracrine secretions led to a synergistic effect strongly upregulating the chemokine SDF1, stemness genes OCT4 and NANOG, and growth factors such as HBEGF. In the mouse model no short-term complications occurred. A foreign body response to the scaffolds was observed in all groups which was modulated by MSC. In the absence of MSC, slender cuffs of mononucleated histiocytes surrounded the implant fibres, with numerous multinucleated giant cells, although neutrophils were rare. In comparison, scaffolds bearing MSC had fewer histiocytes, minimal giant cells, and positive organisation of neo tissue in the open scaffold spaces. Collagen coating of the scaffolds, although increasing MSC adhesion, produced more mononuclear histiocytes and eosinophils surrounding the coated fibres.

Conclusions
Cavity wound scalable medical grade PCL MEW scaffolds seeded with GMP allogenic adult MSC were investigated to facilitate faster translation to the clinic. PCR array results and testing in a complex biological environment confirmed the advantage of using a combination of single and spheroid MSC on non-collagen coated patient ready scaffolds.

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_343 - Biofabrication of a bioactive three-layered skin hydrogel based on ECM components effectively promotes wound healing and regeneration

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Introduction/Objectives
Skin tissue engineering (TE) seeks to develop substitutes for damaged skin, yet conventional methods often lack the complexity needed to fully replicate natural skin's structure and function. To overcome the limitations of conventional skin TE, 3D biofabrication approaches are being developed to enhance biomimicry in skin models. However, most current skin substitutes focus on recreating dermal and epidermal layers while often neglecting the hypodermis, which plays a critical role in epidermal differentiation as well as in providing mechanical and thermoregulatory support. In this study, we developed and characterized biomimetic hydrogels to obtain a biofabricated three-layered (BT) skin substitute based on the main extracellular matrix (ECM) components found in the epidermal, dermal, and hypodermal skin layers, aiming to achieve a closer structural and functional resemblance to native skin.

Methods
Hydrogels for dermal and hypodermal skin layers were based on a mix of agarose and type I collagen, supplemented with skin-related ECM components (dermatan sulfate, hyaluronic acid, and elastin) and loaded with human dermal fibroblasts (hDFs) or human mesenchymal stromal cells (hMSCs), respectively. The epidermal hydrogel was formulated using type I collagen supplemented with keratin and sphingolipids, and seeded with human epidermal keratinocytes (hEKs). After evaluating the diverse physicochemical, mechanical, and biological properties of the hydrogel solutions, we biofabricated a three-layered BT Skin substitute that was characterized *in vitro*, and its full-thickness skin wound healing properties were assessed *in vivo*.

Results
Physicochemical analyses revealed that each hydrogel solution had appropriate viscosity, gelation times, and pH. The BT Skin exhibited favorable swelling and degradation kinetics, with mechanical properties in a similar range of human skin. Both the individual hydrogels and the complete BT Skin model showed high cell viability and stable metabolic activity over a three-week period, maintaining the integrity of the three-layered structure throughout. Additionally, both cell-free and cell-loaded BT Skin demonstrated excellent wound healing capacity in an *in vivo* murine skin wound model, promoting faster wound closure compared to autografting, restoring skin barrier integrity and homeostasis parameters, and supporting effective regeneration of the dermis and epidermis.

Conclusions
This bioactive and biomimetic three-layered BT Skin, comprising epidermal, dermal, and hypodermal layers, has a composition that attempts to mimic the natural ECM of the skin, formulated with the characteristic cells and biomolecules present in each skin layer. This advanced formulation supports wound healing and skin regeneration by restoring homeostasis and barrier function, presenting significant potential for diverse clinical applications in skin TE and wound healing treatments, such as enhancing skin substitutes for burn and chronic wound treatment. Additionally, the BT Skin could serve as a versatile *in vitro* model for studies in skin biology, drug testing, and disease modeling, offering a more complex environment for therapeutic development.

Topic: Tissue-Specific Focus
Subtopic: Wound healing
Type: Oral presentation

TERMIS25_432 - Effects of low intensity pulsed ultrasound on macrophages and keratinocytes as a possible new treatment for chronic wound healing

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Introduction/Objectives
Normally, wound healing occurs in a structured and phased manner, that includes hemostasis, inflammation, proliferation, and remodeling. However, chronic wounds are often stuck in the inflammatory phase, which disrupts the healing progress and results in a persistent, non-healing state. Prolonged infiltration of inflammatory cells, such as macrophages, and persistent release of pro-inflammatory cytokines, impair the ability of keratinocytes to migrate and proliferate effectively. The complex pathophysiology of chronic wounds makes their treatment very challenging, often requiring a multimodal approach that addresses both inflammation and cellular dysfunctions. Despite well-established standards of care, the healing time for chronic wounds often remains prolonged and suboptimal. The use of advanced therapies such as low-intensity pulsed ultrasound (LIPUS) is limited, mostly due to a lack of standard protocols developed to target multiple cell types and molecular pathways. This work aims to systematically screen *in vitro* different LIPUS conditions to decrease inflammation and promote tissue regeneration.

Methods
For this study, THP-1 human monocytes differentiated into M1 pro-inflammatory macrophages with PMA, IFN-γ, and LPS and human epidermal keratinocytes (HaCat) were used. M1 macrophages and HaCaT cells were separately treated with different LIPUS parameters (frequency from 38 kHz to 5 MHz, intensity up to 500 mW/cm^2, duty cycles from 10% to 40%, and different pulse repetition frequencies and durations), by using patented LIPUS in vitro stimulation setup. Multiplex ELISA, immunofluorescence analysis and automated imaging system (OmniFl, Axion Biosystems) were used to assess pro-inflammatory cytokines production, proliferation and migration after LIPUS stimulation. In addition, a stressed phenotype of HaCat cells was generated by culturing them cells in M1-derived conditioned medium for 3 days during scratch assay. Migration and assessment of differentiation markers were analyzed through an automated imaging system and immunofluorescence analyses at the experimental endpoint.

Results
The most effective stimulation conditions were found to be specific for each cell type. LIPUS treatment at 1MHz of frequency, 500mW/cm^2 of intensity, pulsed repetition frequency of 1KHz and duty cycle at 40%, significantly decreased, among others, IL-1β, TNF-α and IL-6 production in M1 macrophages, while HaCat cells stimulated at 1MHz of frequency, 250mW/cm^2 of intensity, pulsed repetition frequency of 10Hz and duty cycle at 10%, significantly increased migration, proliferation of keratinocytes, as well as increased expression of wound-responsive markers KRT16/KRT17 and inflammatory responsive markers HLA-DR and CD146. In addition, preliminary analysis of HaCat cells cultured in M1-conditioned medium showed reduced expression of proliferative markers, including Ki67 and delayed wound healing compared to control, similarly to chronic wound-associated keratinocytes.

Conclusions
These data demonstrate the therapeutic potential of LIPUS treatment for chronic wound healing under specific and previously underexplored exposure conditions. As a perspective, the best LIPUS protocol that simultaneously targets the main cell types involved in the pathology could be translated clinically.

Topic: Tissue-Specific Focus
Subtopic: Wound healing
Type: Oral presentation

TERMIS25_681 - Functionalized bioactive scaffolds for treatment of complex diabetic wounds with bone infection

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Introduction/Objectives
Diabetes worldwide has assumed epidemic proportions with over 650 million diabetics predicted by 2040. Chronic diabetic wounds

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involving bone infections are one of the major factors behind non-traumatic amputations and difficult to manage due to underlying conditions. Pathophysiological complications associated with diabetic wounds include increased oxidative stress, hypoxia, impaired angiogenesis, and microbial growth. Hence, a multipronged approach for clinical usage is needed for treatment of diabetic wounds involving bone infections which can tackle both soft tissue and bone conditions simultaneously along with targeting the associated complications. Nitric oxide (NO) is a free radical and a bioactive molecule. NO initiates the process of vasodilation that provides better blood circulation during wound conditions. In traumatic injuries NO helps in angiogenesis and differentiation/proliferation. Exogenous supply of antioxidants and oxygen has been found useful in mitigating oxidative stress related diseases and hypoxic conditions. To overcome the microbial infections in wounds many broad-spectrum agents such as iodine have been used. Cryogels with their highly porous infrastructure, immense exudate absorbing capacity, free exchange of nutrients and ability to provide niche for different bioactive agents are a potent choice for wound dressings. Hydroxyapatite based bone cements impregnated with antibiotics have immense potential for bone infections associated with diabetic wounds.

Methods
In the present work a dual approach has been applied to tackle skin wounds as well bone infections simultaneously in diabetic conditions. For skin wound, a chitosan gelatin based cryogel with NO, antioxidant, oxygen releasing and iodine for antibacterial property along with exosomes for additional therapeutic property was applied. For bone infection, hydroxyapatite-based bone cement impregnated with rifampicin along with exosomes was applied. The scaffolds were thoroughly characterized followed by *in vitro* and *in vivo* studies.

Results
Characterization results showed highly porous structure of skin scaffolds with pore size ~40-60 μm along with high water uptake capacity and swelling ration. Scaffolds showed sustained release of, skin scaffold- NO, oxygen, iodine and bone cement- rifampicin. The developed scaffolds showed excellent *in vitro* antibacterial, cell migration, biocompatibility, proliferation and osteogenic potential. During the *in vivo* assessment, the scaffolds showed enhanced wound closure, well skin ultrastructure formation, bone formation and reduction in bacterial burden at the bone infection site.

Conclusions
Results have shown significantly improved strategy for advanced diabetic wounds involving bone infections and therefore have the potential for development as a product as per national/international industrial and clinical standards. The functionalized scaffolds will aid in overcoming the healthcare burden posed due to diabetic wounds.

Topic: Tissue-Specific Focus
Subtopic: Wound healing
Type: Oral presentation

TERMIS25_1369 - Development of 3D printed bioactive hybrid dressing using decellularized human amnion bioink for effective management of diabetic foot ulcers and burns

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Introduction/Objectives
In India, diabetic foot ulcers (DFUs) are a significant complication, costing nearly $1,960 to treat, which requires an average of 5.7 years of income for Indian patients. Although exact population-based data is unavailable, it is estimated that around 45,000 legs are amputated annually due to DFUs. Additionally, India represents one of the highest rates of burn incidents. Further, this problem is compounded by infiltration of pathogens leading to heavily exudating wounds (exudate rate 0.87-1.2 g/cm2/day). To solve this problem, we developed 3D printed human amnion-based hybrid dressing for full thickness chronic wounds and second-degree burn (thickness 0.12 mm - 4mm). The amnion bioink layer was printed over silver-based PU foam, serving as microbial barrier and moisture vapour regulator. The bioactive surface was in direct contact with the wound bed releasing prohealing factors. This design allowed wound exudate to pass through the porous bioactive layer and be absorbed by the underlying substrate. The objectives are as follows: (1) Fabrication of 3D printed bioactive hybrid dressing, (2) In vivo assessment of hybrid dressing in infected rodent wound model, (3) Ex vivo assessment of infection control and healed tissue quality.

Methods
Fresh human amnion sourced from Tata Memorial Hospital Tissue bank Mumbai, were decellularized, lyophilised and cryomilled for the bioink preparation. Excipients included A-type skin gelatin, hyaluronic acid and tissue transglutaminase. Printability was optimized through rheology and cell viability was assessed. Amnion bioink was 3D printed over the inhouse developed silver-based PU foam layer, lyophilised and gamma sterilised. Efficacy of hybrid dressing was determined against commercially available silver dressing (Biatain, Coloplast, USA) using rodent wound model infected with *Staphylococcus aureus*.

Results

- In vitro cell viability was assessed by culturing mouse fibroblast cell lines (L929) over the graft for a duration of 20 days. The viability of the cells grown over the graft was recorded as 105.65 ± 5.5 %, 100.11 ± 9.06 %, 89.11 ± 3.70 %, 98.85 ± 4.10 %, 88.95 ± 4.62 % on day 1, 3, 7, 14 and 20 respectively. Notably, cell viability closely resembled that of cells cultured on fresh amnion membrane.
- Average particle size of cryomilled amnion was found to be 31.261±0.158 μm. Powder flow behaviour analysed by shear measurement under the pre shear normal stress of 3 kPa, 6 kPa and 9 kPa with 3 shear-to-failure steps at a constant 0.005 rpm. At all these load conditions, bioactive powder remained to be in free-flowing region. Rheology of bioink demonstrated dominant elastic behaviour and good self-healing property.
- Complete healing was achieved within 15 days, with a marked reduction in viable bacterial count (from full growth to 70±23.15 colonies) and wound closure was comparable to comparator dressing.
- Ex vivo histopathological evaluation of wound biopsy affirmed gradual improvement of healing attributes. It showed increased epithelialization, restoration of rete pegs, collagen basket weave like pattern and CD 31 expression in comparison to comparator dressing.

Conclusions
This hybrid dressing offers effective wound management by combining regenerative, antimicrobial and absorbent properties for managing highly exudative chronic wounds.

Topic: AI, Digitization, Big data
Subtopic: Omics
Type: Oral presentation

TERMIS25_288 - Characterizing the impact of a tridimensional architecture in human engineered cardiac tissues at single cell and spatial resolution using next generation sequencing

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Introduction/Objectives
Developments in the field of tissue engineering and cellular reprogramming have opened new avenues to achieve cardiac regeneration

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and tissue repair. Still, currently one of the main challenges is to attain enough maturation and biomimetism to reproduce the functional and biological aspects of its natural counterpart. Thus, our work aims to transcriptomically characterize our human bioengineered myocardium at single cell and spatial resolution. This will enable the study of the impact of the tridimensional architecture and its differences with respect to published in vivo datasets to further improve the fabrication of myocardial tissues in vitro.

Methods
We performed single cell RNA sequencing (scRNA-seq) with the 10x Genomics Chromium 3′ technology for three biological replicates of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), cardiac fibroblasts (hiPSC-CFs) and hiPSCs-CMs and hiPSC-CFs in a 9:1 proportion either cultured for 30 days in conventional 2D cultures or embedded in a fibrin hydrogel and combined with a 3D printed Melt Electrowriting (MEW) scaffold with diamond-shape geometry. For the tridimensional tissues we also performed spatial analysis considering the Visium CytAssyst and Visium HD technologies. Downstream bioinformatic analyses were mainly performed with Seurat.

Results
A population analysis of the scRNA-seq data from the hiPSC-CMs samples at day 0 demonstrated that most cells had a cardiomyocyte phenotype, though there was a single off-target population (5-10% of cells) that corresponded to fibroblasts, correlating with FACS data. Even if we only retrieved fibroblasts in the hiPSC-CFs at day 0, their transcriptomic profile was distinct from those present in the hiPSC-CMs condition. Interestingly, the fibroblast population in both 2D and 3D samples at day 30 presented a similar expression profile to those coming from the hiPSC-CMs at day 0, hinting to a loss of most of the hiPSC-CF cells during the maturation process. Focusing on the cardiomyocyte population and comparing them to published embryonic datasets in vivo, they were mainly annotated as ventricular or pacemaker phenotypes at nine post conception weeks (pcw), with a few annotated as later stages such as 11 or 13 pcw. Overall, CMs at day 30 presented upregulation of biological processes related to calcium conduction, muscle contraction and oxidative phosphorylation, with 3D minitissues presenting a more glycolytic and hypoxic phenotype and 2D cultures enriched for apoptotic and stress signatures. Finally, an initial dive into the spatial transcriptomic data of the contractile 3D human cardiac engineered tissues pointed to a regular distribution of the different CM populations, though there seemed to be a higher proportion of fibroblasts close to the MEW fibers than within the diamonds.

Conclusions
We provide a first transcriptomic and spatial characterization of a human cardiac engineered tissue, observing features of maturation with respect to the hiPSC-CMs at day 0 and a seemingly low survival of inputted hiPSC-CFs. However, the populations in our tissues still resemble their first trimester counterparts. Thus, further analyses into gene expression changes or transcription factor activities must be performed to find key modifications that will yield advanced maturation of engineered human myocardium with greater mimetism and therapeutic capacity.

Topic: AI, Digitization, Big data
Subtopic: Prediction
Type: Oral presentation

TERMIS25_455 - Identifying optimal hydrogel parameters for intervertebral disc regeneration via an agent-based computational platform

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Introduction/Objectives
Low back pain is one of the world's leading causes of disability, with a healthcare burden that is expected to continue to increase with an aging population. Cell-based biomaterials offer a promising therapeutic strategy to mitigate intervertebral disc (IVD) degeneration by modulating cell behavior in the nucleus pulposus (NP) region, where degeneration usually begins. By varying their chemical composition, biomaterials can be tuned to promote cell growth and regeneration in a tissue-specific context. Compared to in vitro experiments, computational (in silico) studies can help provide a more system-level understanding of key cellular and molecular processes under the influence of physical biomaterial properties, which will in turn allow for the design of more effective, tissue-specific biomaterials. Here, we present a 3D agent-based model (ABM) computing platform that will predict NP cell behaviors within an alginate hydrogel biomaterial.

Methods
The intervertebral disc hydrogel (IVDH)-ABM was built in C++ and represents a 27mm3 hydrogel with 27,000 initially seeded cells. Agents (NP cells) were programmed to follow a set of fundamental rules: migration, proliferation, apoptosis, and extracellular matrix (ECM) & cytokine production, in response to biomaterial elasticity. IVDH-ABM rules were developed using empirical data from the literature, including an equation to calculate hydrogel elasticity from material parameters (alginate molecular weight (MW), alginate polymer concentration, and calcium crosslinker density). Unknown model parameters were optimized using the gradient-free Nelder-Mead technique. The calibrated model was then validated against an independent set of empirical literature data. Model uncertainty was quantified via a bootstrapping method. The verified IVDH-ABM was employed in an in-silico experiment to determine the optimal hydrogel composition (alginate concentration and crosslinker density) for NP regeneration over 21 days.

Results
The IVDH-ABM accurately predicts hydrogel stiffness given material parameter input (values within empirical standard deviation). Synthesis of TGF-β, IL-1β, and TNF-α in the model align with experimental observations; TGF-β accumulation peaked at day 9 of the simulation, whereas IL-1β and TNF-α accumulation peaked at day 4 and stabilized, reflecting their early roles in the inflammatory process. The in-silico experiment identified an optimal hydrogel formulation of 200 kDa-1.95% w/v-0.02 M (alginate MW-alginate concentration-crosslinker density) whose elasticity (3.66 kPa) fell within the physiological range for native NP tissue (5.39 ± 2.56 kPa).

Conclusions
The optimized hydrogel (elastic modulus 3.66 kPa) demonstrated enhanced cell viability (4.01-fold and 4.1-fold increases from two in-vitro experimental hydrogel conditions), proliferation, and ECM synthesis, supporting previous experimental results that found increased aggrecan and collagen production for cells in softer (i.e., lower elastic modulus) hydrogels. This in-silico framework can be expanded to include stem cells, different biomaterials, and even tissue to build a robust "digital microscope" for driving cell-based biomaterials towards clinical application.

Topic: AI, Digitization, Big data
Subtopic: Omics
Type: Oral presentation

TERMIS25_659 - Spatial transcriptomics revealed molecular control of tenogenic and fibrocartilaginous formation in enthesis development

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Introduction
The enthesis, a fibrocartilaginous transition between tendon and bone, is necessary for force transfer from muscle to bone to produce

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joint motion. The enthesis is prone to injury due to mechanical demands, and it cannot regenerate. A better understanding of how the enthesis develops will lead to more effective therapies to prevent pathology and promote regeneration [1].

Methods
The humeral head-supraspinatus enthesis was dissected from C57/BL6 mice at embryonic day 14.5 (E14.5), postnatal day 0 (P0), P7, P14, and P28. Tissues were snap-frozen in liquid nitrogen prechilled isopentane with optimal cutting temperature compound. Cryosections (10 μm) were adhered to Stereo-seq chips for spatial transcriptomics [2]. The enthesis area was circumscribed to extract spatial coordinates from the transcriptomics slides. Datasets from different time points were integrated and batch-corrected using "harmony." Leiden graph-based clustering was performed for unsupervised cell classification, with manual annotation based on marker genes and adjacent H&E staining. Clusters related to fibrocartilaginous and tenogenic differentiation were selected, and pseudotime analysis was conducted using "PAGA" and "SpaceFlow" to visualize spatiotemporal trajectories. Signaling directions were inferred by constructing cell-cell communication using "commot.

Results
Using uniform manifold approximation and projection (UMAP), we identified 14 cell clusters classified into progenitor, tenogenic, chondrogenic, and osteogenic lineages based on marker genes and spatial locations. Two distinct subpopulations of enthesoblasts were characterized: enthesoblasts I, marked by high Col2a1 expression, were located on the bone surface at E14.5, giving rise to UFCs and FCs during later stages (P7–P28). Enthesoblasts II, with high Col3a1 expression, were located adjacent to enthesoblasts I. Tenogenic clusters—including pre-tenoblasts, tenoblasts, and tenocytes—were present during the postnatal period (P0–P28). Both enthesoblast subpopulations were abundant at E14.5 and P0 but were gradually replaced by more differentiated clusters. Pseudotime analysis indicated that enthesoblasts I were more likely to differentiate into mineralizing chondrocytes, while enthesoblasts II were more prone to develop into tenoblasts or tenocytes. Additionally, FGF and GAS were implicated in tendon development, whereas SPP1 and ANGPTL signaling contributed to fibrocartilage generation.

Conclusions
Our findings elucidate the intricate spatiotemporal dynamics of enthesis development. The two subpopulations of enthesoblasts serve as critical differentiation points for enthesis progenitors. Enthesoblasts I give rise to fibrochondrocytes during the late postnatal period in the transitional zone between bone and tendon, while enthesoblasts II are more likely to differentiate into tenogenic cells, contributing to tendon formation from the embryonic stage.

Acknowledgement:
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2. Chen, A., et al., 2022. 185(10): p. 1777-1792.e21.

Topic: AI, Digitization, Big data
Subtopic: Omics
Type: Oral presentation

TERMIS25_673 - Comparative proteomic analysis of the composition of decellularized extracellular matrix (dECM) and dECM-based inks as compared to the native tissue

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Introduction/Objectives
Regenerative medicine and tissue engineering approaches based on the use of 3D-bioprinted decellularized extracellular matrix (dECM) present the advantage of a relatively biomolecule-rich matrix, which directs cell growth and differentiation in a tissue-specific manner. However, little is known about the composition changes that occur with standard processing of dECM-based inks.

Methods
To characterize this process, six porcine tissues/tissue layers (artery, breast, dermis, epidermis, muscle and nerve) were independently decellularized via chemical, mechanical and enzymatic processes and the resulting dECMs formulated into biocompatible inks, to serve as source biomaterials for 3D printing. A comparative liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based proteomic analysis was carried out for native tissue, decellularized and formulated ECMs, and the resulting complexity of the matrisome analyzed.

Results
A core matrisome was found to overlap in all decellularized tissues, as well as tissue-specific components that correlated with predicted functional (gene ontology-based) definitions. The proportion of collagens (mostly the α1 chains of collagen type I and III) increased in the final processing step (inks) as compared to the native ECM and dECM stages. Overall, a median of 55 matrisomal proteins (range 45-126) was detected in the dECM-derived inks. This complexity is far superior in terms of mimicking the composition of native tissue to non-dECM-based inks.

Conclusions
Our results support the use of dECM-based inks and biomaterials in mimicking native tissue ECM complexity.

Topic: AI, Digitization, Big data
Subtopic: Omics
Type: Oral presentation

TERMIS25_748 - Chondrogenesis, Cell metabolism and Spatial biology using the Hyperion Imaging Mass Cytometry system

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Introduction/Objectives
Cellular metabolism plays a determinant role in stem cell function. Progenitor cells adapt their metabolic processes based on their specific needs, whether to maintain a quiescent state, self-renew or initiate differentiation. During chondrogenesis, progenitor cells undergo a dramatic shift in their metabolism to adopt a metabolic profile suited for cartilage tissue formation which is characterized by an avascular environment with limited oxygen and nutrient availability as well as metabolic waste removal. In this study, we used Hyperion Imaging Mass Cytometry (IMC) system to gain unprecedented single-cell insights into the spatial relationships of cells and their phenotypes in the tissue microenvironment to capture their metabolic switch during chondrogenesis *in vitro.*

Methods
The expression level of 22 selected markers related to cartilage, energy producing pathways and quiescence/cell cycle was analyzed concomitantly on progenitor cells derived from (i) Bone Marrow (BMSCs) and (iI) Adipose Tissue (ASCs), as well as de-differentiated cells from different cartilage sources, namely (i) Nasal Septum (NCs) and (ii) Articular Cartilage (CHs). In addition to IMC analyses conducted after 3, 7, 14, 21 and 28 days of 3D cell-culture, engineered cartilage tissues were analyzed (immuno-)histologically and biochemically for glycosaminoglycan contents (GAG) as well as their metabolite consumption/production. After IMC slide acquisitions, Ilastik and Cellprofiler were used for cell segmentation and downstream bioinformatical analyses conducted to generate

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dimension reduction plots and spatial visualisation of the identified cell clusters for more than 750'000 individual cells of 190 3D-engineered tissues.

Results
For all the cell types tested, mature cartilage spheroids were generated attested by Safanin-O staining, GAG quantification and gene expression. The cartilage formation was accompanied with a switch to a more glycolytic profile with an increase in glucose consumption and lactate production, being most pronounced for BMSCs. Whilst the cartilage formation appeared to follow a similar pattern resulting in a comparable quality of cartilaginous tissue, as defined by classical means of analyses, IMC analysis showed stark differences in how the different cell types undergo this chondrogenesis. The spatiotemporal analyses at a single cell resolution revealed the complex diversity of cell states and their localization within each cartilage spheroids. We further identified relevant cell populations contributing to chondrogenesis and defined their metabolic profile. Remarkably, the IMCs analysis of 3D-engineered tissues at day 3 identified specific cell clusters only present in certain donors with good cartilage forming capacity.

Conclusions
This study highlights the importance of spatial biology technologies such as the IMC to get spatiotemporal insights at single cell resolution to better understand tissue development and architecture.

Topic: AI, Digitization, Big data
Subtopic: Artificial Intelligence (AI), machine learning
Type: Oral presentation

TERMIS25_786 - Data quality considerations for AI-based cell image analysis

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Introduction/Objectives
The industrialization of regenerative medicine and tissue engineering continues to progress, with the development and approval of advanced therapy medicinal products (ATMPs). However, ensuring stable and standardized cell culture for ATMP production remains a significant challenge, particularly for maintaining consistent quality throughout the product lifecycle. To address this, integrating cutting-edge automation and artificial intelligence (AI) technologies into cell culture processes is critical. While AI has demonstrated remarkable advancements in other industries, its application in cell culture often overlooks the risks associated with data quality. In fields like cell image AI analysis, where deep learning and transformer models are increasingly applied, the limited availability of high-quality training datasets frequently leads to discussions focused solely on model accuracy rather than data quality. This study investigates how variations in cell image data quality impact AI model performance and demonstrates the benefits of controlled data quality for achieving stable and reliable AI results.

Methods
Over 5,000 time-lapse phase-contrast images were collected, including images of mesenchymal stem cells (MSCs) and other normal cells commonly used in regenerative medicine. These images were analyzed using a comprehensive AI pipeline consisting of image acquisition, segmentation, and feature extraction. The analysis focused on identifying stages in the pipeline where data instability and noise could degrade AI performance. Furthermore, the study examined how data diversity and quality influence the performance of advanced AI models, including deep learning and transformer networks.

Results
Critical risks of noise and bias were identified at every stage of the pipeline, including image acquisition, segmentation, and feature extraction, all of which could lead to significant AI model errors. Additionally, advanced AI models demonstrated unstable performance when trained on datasets lacking sufficient diversity and quality. These findings emphasize the importance of addressing data-related issues to improve AI model reliability.

Conclusions
This study highlights the essential role of data quality and quantity in developing reliable AI models for cell imaging. The results indicate that while AI technology continues to advance, the cell culture field must focus on improving data quality to ensure effective and stable AI performance. Aligning AI models with specific goals and implementing robust data management strategies are critical for advancing AI applications in regenerative medicine and tissue engineering.

Topic: AI, Digitization, Big data
Subtopic: Artificial Intelligence (AI), machine learning
Type: Oral presentation

TERMIS25_867 - Rational self-assembling Peptide Design by Mathematical Modeling for Developing Membraneless Organelle

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Introduction
Membraneless organelles (MLOs), formed through liquid-liquid phase separation (LLPS), can be a transformative strategy for intracellular therapeutic applications in regenerative medicine. We developed a computational pipeline to design short peptides (10 amino acids) for LLPS and functional MLO assembly.

Methods
Our design process employs a multi-objective optimization function:

$$F(P) = \alpha_1 \cdot A + \alpha_2 \cdot H + \alpha_3 \cdot C - \alpha_4 \cdot D$$

where C is the peptide's net charge, H is hydrophobicity, D is the extent of disorder, and A is aggregation propensity. $\alpha_{1\text{-}4}$ are weighting factors optimized to determine the final objective F(P). α_1 facilitates self-assembly while avoiding the formation of non-specific aggregates, α_2 balances hydrophobicity to favour LLPS without impairing solubility, α_3 enhances charge contributions for electrostatic interactions, α_4 penalizes excessive disorder that may reduce structural functionality and compete with phase separation. To address the risk of non-specific aggregation, a penalty function was introduced:

$$\text{Penalty}(P) = \gamma \cdot A^2$$

Where A^2represents the squared aggregation propensity to magnify the impact of high aggregation tendencies, and γ is a scaling factor determining the penalty strength, ensuring selected peptides exhibit dynamic, liquid-like self-assembly characteristic of LLPS. We also applied constraints, including the charge-to-hydrophobicity ratio (B) and solubility index (S(P)), to ensure peptides had optimal profiles for LLPS, which are defined as:

$$B = C/H, \text{ with } 0.05 \leq B \leq 0.15$$
$$S(P) = \beta_1 \cdot C - \beta_2 \cdot H + \beta_3 \cdot MW$$

where $\beta_{1\text{-}3}$ are weighting factors that balance the contributions of charge (C), hydrophobicity (H), and molecular weight (MW) to peptide solubility. B balances electrostatic and hydrophobic interactions for reversible phase separation, while S(P) ensures solubility under physiological conditions, preventing uncontrolled aggregation. Using stochastic sampling, a library of 1,000,000 peptide sequences was generated.

Results
The top 10 candidates were selected based on their F(P) score and penalty adjustments. Low-scoring peptides with unfavorable properties, such as minimal charge and high aggregation propensity, were

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used as controls to validate the scoring process. The obtained data confirmed that these peptides were unable to drive LLPS or form dynamic condensates under physiological conditions, demonstrating the robustness of the model in distinguishing functional peptides from non-functional ones. Through simulations, the selected peptides revealed charge states between +2.03 and +3.03 and hydrophobicity ranging from 40% to 60%, confirming their suitability for LLPS under physiological conditions. The findings demonstrated the formation of dynamic clusters characteristic of LLPS, with the assemblies exhibiting reversible behaviour under varying environmental conditions.

Conclusions
By combining mathematical modeling with de novo peptide engineering, this study demonstrates a robust platform for constructing peptide-based MLOs.

Acknowledgement
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Topic: AI, Digitization, Big data
Subtopic: Prediction
Type: Oral presentation

TERMIS25_1198 - Computational prediction of the oxygenation of a living chicken embryo developing inside a synthetic eggshell platform

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Objectives: Recent advances [1] remarked the importance of understanding angiogenesis, essential for the development of new antiangiogenic, antibody-based, and small molecule-based drugs in the field of cancer research. To accelerate those studies and the relevant preclinical testing of new drugs, particularly those targeting the microcirculation, also protagonist in severe conditions like diabetes, retinal and kidney diseases, and viral infections, we urgently need more efficient and effective preclinical models. The chick embryo is an ideal model for conducting rapid and adaptable studies on how a functional vascular network responds to various stimuli. In this work, we developed a computational model of the embryo across different developmental stages to identify the best synthetic material and geometry capable of replacing the natural eggshell while maintaining physiological oxygenation. We realized this eggshell and experimental results confirmed our predictive model efficacy.
Methods: To depict a live chicken embryo along with its development and to describe its own protective shell, we set up computational simulations using COMSOL Multiphysics. We modeled the egg as a prolate ellipsoid made of a porous material with a thickness of 300μm and porosity <2%. We assumed the cuticle thickness of 65μm. We hypothesized the embryo located in the center of the egg. We assumed a consuming surface of about 41cm^2 for the chorioallantoic membrane, which is the respiratory organ of the embryo. The peak oxygen consumption, or V_{max}, assumed was 5.14×10^{-6}mol/m^{2}s, following the Michaelis-Menten kinetics. Next, for creating a substitute of the eggshell, we considered several silicone substitutes having thick of 100, 365, 800μm (limit). We modeled an innovative geometry to maximize the O_2 flux without reducing the membrane thickness excessively close to the limit value.
Results: Physiological eggshell simulation described the embryonic behavior in the natural eggshell with an oxygen prediction error lower than 1% with respect to our experimental measurements. In the synthetic eggshell model, the mean oxygen content in the air sac experienced a linear drop in the first 8 days of embryo growing with a final value of 4.2mol/m^3. By analyzing the lateral lining concentration profile, simulations predicted a drop in air sac concentration ranging from 25% to 80% with respect to values known for the natural eggshell. In vivo assessment of the oxygen level confirmed our simulated predictions. Viability tests (n>3) provided further confirmation of our numerical results.
Conclusions: We developed a computational model to predict the oxygen concentration levels in a chicken embryo on its 12th day of development. Thanks to precise model calibration, we were able to reproduce the experimental O_2 measurements reported in the literature [2]. Our synthetic eggshell platform paves the way to growing avian embryonic bodies in a well oxygenated environment.

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Topic: AI, Digitization, Big data
Subtopic: Modelling disease
Type: Oral presentation

TERMIS25_1349 - Key features of invasive breast cancer cell phenotypes: a data-informed approach designing in-vitro experiments using micro-physiological systems

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Introduction
Microphysical systems (MPS) are in-vitro models that provide advanced approaches to replicate the complexity of human tissues outside the body by providing more relevant microenvironments compared to traditional 2D cell cultures. Based on our previous studies, we herein engineered a 3D MPS model of breast tissue using tumour microenvironment (TME) mimetic in-vitro systems with well defined, reproducible, and customizable properties to match the breast-specific microenvironment. In specific, we were able to independently control physico-chemical properties of the extracellular matrix with tailor-made hydrogels, environmental pH and fluid flow. To assist the interpretation of the large amount of experimental data generated from these experiments, we report on how the use of advanced analytical tools is required to drive data informed decisions on simplifying experimental design and resultant data analysis.

Methods
Two breast cancer cell lines were used: the highly invasive triple negative cancer cell line MDA-MB 231 and the less invasive luminal type MCF-7 cell line. The cells were cultured in Alginate-gelatin hydrogels which were engineered to have varying matrix stiffness in the range of 1-10 kPa. The cell culture was kept either at physiological pH 7.4 or tumorigenic acidic pH 6.5. The interstitial fluid flow was mimicked by culturing the cells in a millifluidic system with a constant rate of media flow (500 μL/min). We measured proliferation, EMT and B-CSC markers as these are all implicated to play a role in breast cancer progression especially in metastatic invasion. All data were organized for further analysis.

Two different paths were used to characterize inter-phenotype and intra-phenotype behaviors. We used K-means to cluster features, the silhouette index to evaluate group separation and an estimate of the mutual information was finally used to rank features allowing for inter-phenotype analysis. To analyze intra-phenotype behavior, K-means was once again exploited in combination with the silhouette index to determine whether distinct behaviors matched tissue-specific microenvironments.

Results
With unsupervised k-means clustering and feature extraction we identified that each microenvironment had a different set of important features that distinguished between the two cell lines. Some microenvironments like acidic pH, perfusion and soft matrix created more differentiating features between invasive and non-invasive phenotypes; suggesting that these micro environmental conditions could be used to validate clinical data. We extracted features that were absolutely necessary to distinguish MDA-MB231 vs MCF-7 in

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each microenvironmental condition. The further analysis returned that mesenchymal markers (Vimentin, CD44 and CD44v6) were the most important features in all microenvironments, hence emphasizing that amount of M-CSCs could be a better differentiating factor for invasion and recurrence than E-CSCs and proliferation.

Conclusions
This study shows a multidisciplinary approach combineing bioengineered breast-specific microenvironments with data drive models to investigate the effect of TME on invasive potential and/or recurrence risk in breast cancer. We report on a unique and innovative platform for studying critical cellular processes in diseased states and pathological context like cancer, which can be further extended to undertand biological processes combining control systems and bioengineering.

Topic: Biomaterials
Subtopic: Biomimetic, multiphasic, or smart models
Type: Oral presentation

TERMIS25_757 - Electroconductive and Adhesive Hyaluronic Acid-Graphite Hydrogel for Spinal Cord Injury Repair

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Introduction/Objectives
Spinal cord injury is a life-altering condition that disrupts neural electrical conduction, leading to severe and often irreversible motor and sensory deficits. Current therapeutic strategies fall short of fully restoring neuronal function, emphasizing an urgent need for advanced neuroregenerative materials able to bridge the damaged nerve pathways and restore neural connectivity and function. Herein, we developed an adhesive and conductive hydrogel by modification of hyaluronic acid (HA) with catechol groups and combining it with exfoliated graphite nanoplatelets (EG) or functionalized EG (f-EG) to enhance the mechanical strength, conductivity, and neurodegenerative potential.

Methods
Catechol-conjugated HA (HA-C) was synthesized through a one-pot reaction of HA-aldehyde with dopamine followed by reduction. The f-EG was produced via a Diels-Alder cycloaddition reaction at 250°C for 3 hours [1]. To produce the hydrogels, HA-C (5% w/v) was dissolved in phosphate buffer saline (pH 8), followed by the addition of NaIO4 (0.1% w/w) and EG or f-EG at concentrations of 1% and 50% w/w. A hydrogel without EG or f-EG was also prepared and used as a control. The mechanical properties and self-healing ability were evaluated using rheological measurements. The conductivity was measured using Ohm's law by applying a controlled voltage of 0.5 V across the hydrogel and recording the resulting current. Then, SH-SY5Y cells (1 x 106 per sample), an undifferentiated neuroblastoma cell line, were cultured on top of the hydrogels, and an electrical stimulation was applied by a Digilent waveform generator (biphasic square wave, 1 Hz, 250 mV). The simulation was performed daily for 1 h during a period of 3 days while maintaining the cultures in a CO_2 incubator. Control samples without simulation were also studied for comparison.

Results
The developed hydrogels demonstrated adhesion to rat spinal cords, maintaining stability even under gravity. Moreover, the gels exhibited self-healing ability and mechanical strength suitable for neural tissue applications ($G' \sim 100$ Pa). The incorporation of 50% f-EG significantly enhanced the conductivity from 0.00086 ± 0.00027 S/m to 0.1 ± 0.09 S/m. The electrical stimulation of SH-SY5Y cultures on HA-C/50% f-EG hydrogels improved cell attachment and viability, as confirmed by ATP quantification and live/dead assay, and increased the neuronal marker β-III tubulin expression.

Conclusions
The HA-C hydrogels reinforced with EG or f-EG demonstrate a higher conductivity, compared with HA-C hydrogel, while maintaining adhesive and self-healing properties. These characteristics resemble the mechanical and electrical properties of native spinal cord tissue, making the developed hydrogels promising candidates for the treatment of spinal cord injuries by bridging damaged neural pathways and supporting neuroregeneration.

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Topic: Biomaterials
Subtopic: Biomimetic, multiphasic, or smart models
Type: Oral presentation

TERMIS25_1118 - Impact of glycosaminoglycan (GAG) variants on myogenesis in 3D, aligned, and conductive collagen-GAG (CG) scaffolds

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Introduction/Objectives
Biomaterial-based tissue engineering shows promise for addressing traumatic volumetric muscle loss (VML) injuries. Recent research has focused on developing biomaterial scaffolds mimicking the 3D, highly aligned, and electrically excitable nature of native muscle, though fewer studies incorporate GAG-mediated biochemical cues. GAGs, linear polysaccharides present in extracellular matrix (ECM), regulate myogenesis and growth factor sequestration. Our lab previously showed enhanced myogenesis in collagen-chondroitin sulfate scaffolds containing hyaluronic acid-doped poly(3,4-ethylenedioxythiophene) (PEDOT) particles compared to non-GAG-doped polypyrrole (PPy)[1], however we did not systematically investigate the combined influence of GAG and conductive polymer (CP) type on myogenesis. This research advances previous work by developing scaffold systems incorporating GAGs in the collagen matrix and conductive CG scaffolds by incorporating GAG-doped CPs, recapitulating native skeletal muscle ECM architecture via freeze-drying to study the combined effects of GAG type and conductivity on myogenesis.

Methods
Collagen suspensions (1.5 wt/v %) with GAGs (0.1 wt/v %) in acetic acid were made and pipetted into a thermally mismatched mold to allow unidirectional heat transfer. Scaffolds with aligned pores were obtained by freeze-drying collagen suspensions containing hyaluronic acid, chondroitin sulfate, and heparin with increasing sulfation levels. Following dehydrothermal treatment and carbodiimide crosslinking, scaffolds were seeded with myoblasts. Cell metabolic activity was assessed using an AlamarBlue assay over a culture time of 4 days in growth media and 7 days in differentiation media. For immunocytochemistry, scaffolds were stained for nuclei (DAPI), F-actin, and myosin heavy chain (MHC). GAG-doped conductive PPy and PEDOT particles were synthesized via chemical oxidative polymerization using ammonium persulfate and respective GAGs.

Results
Scanning electronic microscopy (dry state) revealed a similar, microporous structure (diameter $\sim$ 250 μm) across all 3 scaffold

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groups while pore orientation angle analysis revealed aligned pore structure in longitudinal plane. Orientation analysis of F-actin staining showed cellular alignment confirming to scaffold structure. Tensile testing of dry scaffolds resulted in Young's moduli value ~ 1 MPa and the scaffolds were mechanically stable when hydrated. Notably, the heparin-incorporated group demonstrated a significant increase in cell metabolic activity fold change ($n = 4$, $p < 0.005$, $p < 0.0005$). Additionally, the heparin-incorporated scaffolds exhibited enhanced MHC-positive cells, characterized by elongated, multinucleated structures, compared to other groups.

Conclusions
We successfully engineered CG scaffolds with an anisotropic structure via freeze-drying to mimic native skeletal muscle as a platform to repair VML. The 3D pore alignment has provided contact guidance to align cells. Studies have demonstrated that the higher sulfation levels of heparin enhance growth factor sequestration, which aligns with our findings: heparin integration resulted in significantly improved cell adhesion, proliferation and differentiation compared to other groups. Ongoing work is evaluating the impact of GAG-doped CP particles on myogenesis and conductivity within conductive scaffold systems.

[1]Basurto J Biomed Mater Res. 2022

Topic: Biomaterials
Subtopic: Biomimetic, multiphasic, or smart models
Type: Oral presentation

TERMIS25_1380 - Advanced Conductive Bioinks for Cardiac Tissue Engineering

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Introduction/Objectives
Cardiovascular diseases remain a leading cause of mortality, highlighting the urgent need for innovative regenerative therapies. This study aimed to develop advanced bioinks that replicate native cardiac tissue properties, integrating conductive materials to promote cardiomyocyte (CM) maturation and function.

Methods
GelMA (gelatin methacrylate)-based bioinks were enhanced with MXenes and carbon nanotubes (CNTs) to create an electrically conducive environment. These bioinks were subjected to comprehensive characterization, including conductivity, rheological properties, and printability, followed by cell viability and metabolic activity assessments. Electrical stimulation was applied to evaluate its impact on CM alignment and maturation.

Results
Both MXene- and CNT-enhanced bioinks demonstrated superior conductivity and mechanical stability, supporting CM growth without compromising cell viability. Electrically stimulated constructs showed increased metabolic activity and organization, although 3D bioprinted CMs remained immature in shape. The incorporation of MXenes further facilitated electrical signal propagation, enhancing CM contraction and maturation, critical for functional cardiac tissues.

Conclusions
This study underscores the importance of conductive bioinks, particularly GelMA-MXene formulations, in advancing cardiac tissue engineering. These bioinks not only improve the electroconductive properties of 3D-printed cardiac tissues but also provide a scalable and biocompatible solution for regenerative medicine applications. By mimicking the natural cellular environment, this approach offers potential for cardiac repair and broader bioelectronic applications.

Topic: Cancer
Subtopic: Tumor biology
Type: Oral presentation

TERMIS25_153 - Purification of colon cancer cells using membrane filtration method via modified porous polymeric membranes

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Introduction/Objectives
Colorectal cancer (CRC) is a prevalent malignant tumor worldwide, and cancer stem cells (CSCs) play a crucial role in its progression. Despite advanced treatment of patients with CRC, their therapy faces challenges due to CSC resistance to chemotherapy, leading to recurrence and metastasis of the cancer. This research aims to isolate cancer stem cells (CSCs) from the HT-29 colon cancer cell line, which serves as primary models for colorectal cancer (CRC) as well as primary colon cancer cell lines established in our laboratory. We developed two types of modified PLGA membranes for purification of CSCs using the membrane filtration method: one membrane blended with a negatively charged poly(vinyl alcohol-co-itaconic acid) (PVA-IA) and another membrane blended with a positively charged poly(L-lysine) (PLL). The aim of this study is to establish a reliable and effective method for enriching CSCs of colon cancer cells via membrane filtration.

Methods
The colon cancer cells were detached from culture dishes and then the cell suspension solution permeated through the membrane. After collecting the permeation solution, we inverted the membrane and the medium solution was filtered through the membranes to collect the recovery solution. Subsequently, we removed the membrane, located the membranes in cell culture dishes, and cultivated the membranes in cell culture medium, a process referred to as membrane migration. Finally, we assessed the cellular characteristics using a colony formation assay for permeation, recovery and migration solution, as along with the evaluation of CD44 and CD133 (CSC marker) expression using flow cytometry. Subsequently, the membrane filtered solution was evaluated for the production of carcinoembryonic antigen using ELISA kit.

Results
The expression of CD markers on the cells indicated that the CD44 expression on HT-29 cells and patient colon cancer cells after filtration via PLL-modified PLGA membranes were significantly higher than that on unfiltered cells, suggesting an enhanced ratio of CD44-positive cells by membrane filtration of the cells. Similarly, the expression of CD133 on the cells were also increased after the filtration of the cells through PLL-modified PLGA membranes, indicating an enhanced CD133-positive cells by membrane filtration of the cells. The colony formation assay indicated that HT-29 cells and patient colon cancer cells exhibited strong colony formation abilities in the permeation solution after filtration through PLGA (unmodified) membrane. In contrast, the cells filtered through PLL-modified PLGA membranes demonstrated strong colony abilities of the cells in the recovery solution. These results indicated that the cells filtered through specific membranes enhanced clonogenic potential of the cells.

Conclusions
After filtration of cells through PLL-modified PLGA membrane, we effectively enriched cancer stem cells (CSCs) in the recovery solution, as evidenced by the increased expression of CD44 and CD133 markers. The colony formation assay further confirmed the enhanced clonogenic potential of cells in the recovery solution after filtration through PLL-modified membranes. This suggests that PLL-modified PLGA membranes provide a promising method for CSC purification in colon cancer. Efficient purification of CSCs will support the development of effective treatments for patients with colon cancer in the future.

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Topic: Submit to SYMPOSIUM
Subtopic: Biomaterials design strategies for tissue engineering
Type: Oral presentation

TERMIS25_362 - Controlling microenvironments for specific biological functions utilizing chemically modified hyaluronic acid

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Introduction/Objectives
Despite advancements in 3D bioprinting and implantable biomaterials, versatile materials are still needed for diverse musculoskeletal applications. Developing functional biomaterials remains complex, as they must possess a finely tuned balance of physical and biological properties, such as degradation, adhesion, and immunomodulation, while ensuring printing accuracy, cellular viability, and proliferation. Hyaluronic acid (HA) is widely used in these applications. Our objective was to explore chemical modifications of HA to produce biomaterials with controllable properties.

Methods
In the current work we firstly evaluated the capability of macrophage (MΦ) polarization of tyramine-modified hyaluronan (THA) of two molecular weights: M_w = 280 kDa (Low M_w THA) and M_w = 1640 kDa (High M_w THA), supplemented in cell culture medium at 2 mg mL^{-1} concentration, as a soluble signal.[1] The successful THA synthesis was confirmed using ^{1}H-NMR and UV-Vis spectroscopy. We demonstrated the M1/M2 macrophage polarization effects of the supplemented soluble THA at 2 mg mL^{-1} by the semi-automated image analysis from confocal microscopy, immunofluorescent staining utilising CD68 and CD206 surface markers, RT-qPCR gene expression analysis, as well as using the enzyme-linked immunosorbent assay (ELISA). Subsequently, to generate selection of hydrogels with tuneable degradation and adhesion properties, we synthesised oxidized tyramine-modified hyaluronan (o-THA) using sodium periodate $NaIO_4$ with varied molar ratios and evaluated reaction by ^{1}H NMR and UV absorbance measurements utilizing Purpald reagent. We then utilized Ruthenium and sodium persulfate cross-linking methods to generate a selection of hydrogels with variable physicochemical properties.

Results
The degree of substitution (DoS) of tyramine on THA was found to be 6.0 ± 0.6 %. Flow cytometry (on HLA-DR, CD105, CD206, and CD163) and and multiplex ELISA (on thirteen inflammatory cytokins) showed that LMW THA induces a pro-inflammatory M1-like phenotype in MΦs, while HMW THA led to a mixed M1/M2 profile, suggesting a heterogeneous or transitional state. Selection of oxidized THA (o-THA) was synthesized with three degrees of oxidation: 12.6 ± 1.4 %, 17.8 ± 1.8 % and 21.5 ± 2.4 %. Utilizing selection of Ruthenium (0.1- 1 mM) and Sodium persulfate (1-5 mM) concentrations, we prepared an array of hydrogels, whose viscoelastic properties were evaluated by oscillatory rheology and adhesion by lap-shear tests using commercially available Chamonix leather as a model.

Conclusions
The tendency of LMW HA to display inflammatory properties was confirmed in the tyramine derivative, indicating that at this low % of modification pristine HA biological properties are preserved in these biomaterials. Storage modulus of o-THA decreased with oxidation level but was unaffected by molecular weight in photo-cross-linked hydrogels. Higher oxidation yielded faster degradation (<24 hours). We present a family of THA and o-THA biomaterials with tunable physicochemical and biological properties.

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[1] J. K. Wychowaniec, E. I. Bektas, A. J. Vernengo, M. Mürner, M. Airoldi, P. S. Tipay, J. Sapudom, J. Teo, D. Eglin, M. D'Este, *bioRxiv* **2024**, 2024.01.11.575241.

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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_435 - Towards robust biofabrication: In-situ quality assessment in embedded bioprinting with integrated imaging and software analysis

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Introduction/Objectives
Despite significant advances, 3D bioprinting technologies face reproducibility challenges that hinder their deployment in clinical or industrial settings. These challenges arise from the open-loop nature of current bioprinting systems, which lack the ability to monitor extrusion outputs. The primary objective of this study is to improve the reliability and reproducibility challenges of bioprinting processes by developing a bioprinting platform with integrated in-situ monitoring of extrusion quality. This will facilitate the production of high-quality, reproducible outputs that adhere to Good Manufacturing Practice (GMP) standards.

Methods
We developed a novel bioprinting platform integrating a high-resolution camera for in-situ monitoring of extrusion outcomes during embedded bioprinting. Using classical computer vision and image analysis, we created custom software to assess print quality, enabling quantitative comparison of printer outputs to input points of the CAD model 2D projection, measuring area and positional accuracy. To showcase the platform's capabilities, we investigated various inks, bioinks, dyes, and support materials, demonstrating cell viability and ink visibility in 2D and 3D path trajectories. Additionally, we studied how the rheological properties of granular support hydrogels impact print quality during embedded bioprinting, illustrating a practical application of the equipment.

Results
The integration of the novel camera setup and image analysis enabled comprehensive insights into embedded bioprinting processes and the particle-particle interactions in granular support hydrogels with viscosity ranges between 10 to 1000 Pa·s. Our results demonstrated that lower viscosity, faster thixotropy recovery, and smaller particle sizes significantly enhance print fidelity in embedded bioprinting. Fluorescent microscopy was used to track multiple layers, allowing targeted imaging at different depths when lower layers obstructed the process. This approach enabled detailed monitoring of complex multilayered prints.

Conclusions
We showcased the platform's ability to assess key print quality metrics (area and positional accuracy) during bioprinting process development, providing users with advanced quality assessment data that can be used to optimise workflows. This novel bioprinting platform, equipped with integrated process monitoring, holds great potential for establishing robust, reliable, and auditable biofabrication processes for industrial applications.

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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_555 - Creating consistent and reproducible collagen droplet-in-droplet models for tissue engineering

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Introduction/Objectives
The droplet-in-droplet (DiD) geometry is used for 3D models that mimic multilayered tissues in a miniaturized form, suitable for multi-well plates. These models feature a central core droplet encapsulated by an outer droplet. By varying bioink concentrations and crosslinking methods, diverse cellular microenvironments can be generated, including variations in stiffness to mimic specific cell conditions. This study aims to demonstrate the precision, accuracy, and consistency of encapsulating collagen droplets of different sizes in a DiD geometry using extrusion bioprinting. Additionally, we evaluated the model's potential as a proof of concept for cellular migration/ invasion, one of the key applications of the DiD geometry.

Methods
DiD models were created using TeloCol®-10, type-I bovine collagen, neutralized and diluted to the concentration of 6 mg/mL for inner droplets and 4 mg/mL for outer droplets. The BIO ONE bioprinter, equipped with a temperature-controlled syringe-pump printhead, was used for printing. Inner droplets were printed in 6- and 48-well plates, followed by outer droplets. DiDs geometries were created in different setups: arrays (triangular) of 3 DIDs in 6-well plates or single DIDs in 48-well plates. Different volume proportions were used: 5/45 μL for 6-well plates and 5/30 μL for 48-well plates. Constructs were incubated at 37°C for 10 minutes between layers. Imaging was performed using plate scanning, macro photography, and microscopy.

For the cellular migration and invasion study, we used 4x106 cells/mL of MDA-MB-231 mCherry-tagged breast cancer cells mixed with 6 mg/mL collagen for the inner droplet, while the outer droplet composition remained unchanged. These cells are commonly used in research due to their high migratory capacity. To enhance visualization, we used a 1/5 μL proportion of inner to outer droplets and captured images every 3 hours using the CELLCYTEX live cell imaging microscope over a period of 9 days.

Results
Microscope images showed consistent alignment between the inner and outer droplets across wells in the 48-well plate. In the 6-well plate, the alignment was even better, likely due to the larger volume of the outer droplet. The BIO ONE bioprinter ensured precise droplet placement, which is crucial for fully encapsulating the inner droplet within the outer droplet.

By varying hydrogel concentrations in the inner (6 mg/mL) and outer layers (4 mg/mL), we can mimic the stiffer inner core of a tumor, from which cells migrate to the softer, healthier surrounding tissue. In this proof of concept, initial migration of MDA-MB-231 cells from the inner droplet to the cell-free outer droplet was observed after 3 days, followed by significant migration over the subsequent period.

Conclusions
The BIO ONE bioprinter, with its temperature-controlled syringe-pump printhead, demonstrated high precision and consistency in creating DiDs with varying collagen concentrations. Full encapsulation was achieved in both 48- and 6-well plates, using volume combinations of 5/30 μL and 5/45 μL, respectively. We have shown that, these versatile DiD models are valuable for applications in cell migration and invasion studies, making them a useful tool for drug discovery.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_805 - Multifunctional hand-held 3D biopen system for in situ biofabrication onto living tissues

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Introduction/Objectives
The increasing demand for precise, rapid, and versatile tissue regeneration tools has inspired the development of *in situ* bioprinting, leading to handheld devices known as biopens that bring biofabrication capabilities directly into the hands of clinicians. Biopens are emerging as valuable biomedical tools in clinical settings, allowing for real-time, patient-specific deposition of biomaterials or bioinks onto living tissues, addressing the limitations of traditional tissue grafting. Their design enables adaptation to various tissue types at a lower cost than conventional robotic devices. Our project began with an innovative dual-head airbrush designed to spray a novel biomimetic bioink composed of fibrinogen enriched with glycosaminoglycans (GAGs) and collagen (Col) for skin applications. Building on this, we developed a multimodular biopen capable of both spray and extrusion applications, with interchangeable heads for dual-material deposition and broad bioink adaptability across multiple tissue types.

Methods
The initial airbrush was designed with two spray heads for simultaneous dual-material delivery, using an adapted novel fibrinogen/GAGs/Col bioink loaded with human mesenchymal stromal cells or dermal fibroblasts. This bioink was analyzed *in vitro* for its physicochemical and mechanical properties, as well as cell viability, metabolic activity, and wound healing capacity, in comparison to a fibrinogen-only bioink. Finally, its skin wound healing properties were studied *in vivo* in an excisional wound healing murine model. Expanding on these findings, we validated the multifunctional biopen *in vitro* for both spray and extrusion applications. For spray, we used the fluid fibrinogen-based bioink from the airbrush, optimizing spraying pressure to maintain cell viability and metabolic activity. For extrusion, we tested more viscous bioinks, measuring rheological and mechanical properties, along with cell viability and metabolic activity, and compared printability to that of a conventional bioprinter.

Results
The fibrinogen/GAGs/Col bioink applied with the airbrush formed hydrogels with satisfactory physicochemical, mechanical, and biological properties, promoting wound healing and tissue regeneration *in vivo*, with outcomes comparable to autografts. The biopen device maintained cellular viability and stable metabolic activity *in vitro*, with both spray and extrusion bioinks exhibiting optimal mechanical characteristics. Extrusion tests confirmed that the biopen achieved printability metrics comparable to standard 3D bioprinting, highlighting its versatility for multiple tissue engineering applications.

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Conclusions
Both the dual-head airbrush and advanced multifunctional biopen offer promising, versatile solutions for clinical wound care, enhancing the precision and efficiency of skin tissue engineering approaches. The biopen's ability to deliver bioinks via spray and extrusion enables rapid, personalized, ambulatory treatment of skin wounds, reducing hospital stays and facilitating targeted care in clinical settings. These findings underscore the biopen's potential for diverse *in situ* clinical applications – from chronic wound care to burn treatment, to additional tissues such as cartilage and cornea – paving the way for minimally invasive procedures.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_823 - Screening hydrogel composition effects on cell responses using a custom-designed bioprinting mixing tool

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Introduction/Objectives
The precise identification of hydrogel compositions capable of inducing specific cellular responses is essential for exploring the effect of the 3D microenvironment on cell behavior with applications in basic research, drug development and regenerative medicine. This study aims to develop a screening method to identify cell responses to hydrogels composed of well-defined concentrations of components of interest, such as extracellular matrix (ECM) proteins, growth factors, or therapeutic agents. A bioprinting mixing tool was designed for precise control of hydrogel mixing allowing for the creation of arrays of hydrogel composition gradients to investigate how different cell responses are influenced by specific hydrogel properties.

Methods
A bioprinting tool capable of mixing two hydrogels at varying ratios was employed to create a "landscape" of hydrogel compositions. The mixing of hydrogels was performed using an active mixing tool, which was operated using a customized G-code running on our open-source bioprinter[1]. The tool is composed of 3D printed parts, and holds a central mixing chamber where the two incoming hydrogel streams are delivered from separate channels and mixed by the rotation of a central mixing rod. In the current configuration, two independent piston-driven extrusion tools are utilized to control the simultaneous injection of precise volumes of the respective hydrogels into the mixing chamber. The hydrogel mixtures are sequentially dispensed in small volumes through automatized displacement control of the mixing tool in the x, y, z printer coordinate system optimized for a 384 well plate format. The exact hydrogel composition in each well is estimated using fluorescent proxies, which are incorporated into the hydrogels prior to mixing. This workflow is compatible with cell work, supporting both cell culture beneath the hydrogel mixture and cell encapsulation within the hydrogels before mixing. Cell responses to distinct hydrogel mixtures were assessed via microscopy using stains for various cell markers. Fluorescent imaging data were analyzed using Cell Profiler and ImageJ software.

Results
The mixing tool enabled the formation of a hydrogel concentration gradient, facilitating the screening of specific concentrations of components of interest on cell behavior. Gradients of ECM proteins and therapeutic agents mixed in the hydrogel were successfully printed. Cells encapsulated in the hydrogels prior mixing survived passage through the active mixing rod in the mixing chamber. Discrete hydrogel compositions, as tracked with fluorescent proxies, could be associated with specific cell responses.

Conclusions
The custom-built mixing tool facilitates the generation of a screening platform for identifying hydrogel composition effects on cell behavior, enabling exploration and customization of a wide range of potential properties for in vitro 3D models using hydrogels.

[1]Engberg et al. 2021. *Scientific Reports.* doi:10.21203/rs.3.rs-653725/v1.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1443 - Hand-held biopen for "in-clinic" oral tissue regeneration

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Introduction/Objectives
The repair of oral and maxillofacial hard and soft tissue defects is challenging, and there are various problems associated with current therapeutic modalities commonly used in clinical practice, resulting in suboptimal regeneration, long healing time, and poor quality of newly formed bone.

Establishing new bioprinting in-clinical process to control the use of multiple biomaterials and cells will be a key point to rebuild complex soft and hard tissue structures. In terms of manufacturing the key issue is to maintain a good processability while minimizing their impact on cell viability. This will improve repeatability, a critical point for standardization, and multi-material handling and bioprinting capabilities. Such a novel bioprinting technologies will also push the existing biomaterials towards new properties and capabilities.

The main objectives of the work are:

1) Enable technological transfer in biomaterials by developing innovative products with tailored components and functionalization for oral tissue regeneration.
2) To enable the In-situ soft and hard tissue repairing by hand-held bioprinting.
3) Adapt the hand-held bioprinting methodology to the context of in-clinic printing with prototype development.

Methods
The development of the biopen and the biomaterials was divided into 4 well-defined phases:

Phase 1: Biopen Research and Design. Study of compatible biomedical materials. Research on extrusion technologies. Preliminary design of the biopen.

Phase 2: Biopen Development. Manufacturing of the biopen (body, extrusion system). Integration of the UV curing system. Adjustment of components and parameters.

Phase 3: Testing and Validation of the biomaterial. Extrusion and curing tests. Evaluation of mechanical properties of the applications. Evaluation of biological characteristics. Adjustments according to the results obtained.

Phase 4: Evaluation of oral tissue printability in patients with the biomaterials developed by the Emilia-Romagna partner for oral tissue regeneration (alveolar defects and jaw defects). Cell viability.

Results
The developed biopen allowed the implementation of two customized procedures:

i) For focal defects, the treatment was implemented directly into the patient's alveolar lesion with a minimally invasive surgical procedure. The hydrogel osteoinductor will be loaded with the patient's own cells into the Biopen appropriately designed for direct injection into the bone lesion.
ii) For large bone defects, the hydrogel developed to aid bone regeneration was 3D printed using the biopen and freeze-dried, resulting in a dry, mechanically competent, bioactive and bioresorbable porous structures.

Conclusions
The bioprinting methodology will achieve a perfect-fit patient-tailored graft/implant according to the defect, with an adjusted inner

architecture and outer shape to maximize tissue mimicry, will result in functional as well as aesthetically pleasing tissue restoration.

Topic: Bioprinting & Biofabrication
Subtopic: Bioinks
Type: Oral presentation

TERMIS25_523 - Critical Shear Stress: A Novel Metric for Predicting Cell Viability in Biofabrication and Therapeutic Applications

Mehdi Maleki[1], Celine Thomann[1], Elliott Cowles[1], Albane Carré[1], Edwin Courtial[1], Emma Petiot[1]

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Introduction/Objectives
Understanding the impact of hydrodynamic shear stress on cells is crucial in bioprinting and bioprocesses, as it influences cell viability, quality, and cell recovery yields. This study investigates cell tolerance to shear stress to optimize bioprocesses for personalized medicine applications, particularly in cell therapy and tissue engineering via bioprinting.

Methods
A microfluidic system was developed to simulate hydrodynamic conditions representative of bioprocessing/bioprinting environments. This setup, paired with cytometry-based cell quality assessments, allowed for precise quantification of cell viability post-exposure to varying levels of shear stress. Thirteen cell types, including stem cells, differentiated primary cells, cancer cell models and continuous production cell lines, were screened to determine their respective shear stress tolerances. The study introduced the concept of Critical Shear Stress (CSS), a threshold representing the maximum shear level a cell population can withstand while maintaining 80% viability.

Results
Each cell type demonstrated a unique CSS, indicating distinct tolerances to hydrodynamic stress. These findings suggest CSS as a potential critical quality attribute (CQA) for cells used in bioprocesses, allowing for more precise control of cell viability and quality under shear-intensive conditions like micro-extrusion or inkjet bioprinting. The study's results revealed that identifying and respecting each cell type's CSS can enhance cell survival and quality, thereby improving overall process yield.

Conclusions
This study advances biofabrication optimization by introducing CSS as a quantifiable, predictive parameter in cell viability/ cell recovery management. The findings highlight the role of CSS in identifying best parameters to optimise biofabrication efficiency, advocating for predictive control frameworks over traditional trial-and-error approaches. Establishing CSS as a CQA in cell-based hydrodynamic bioprocesses could facilitate more refined design, calibration, and validation practices in therapeutic and tissue engineering applications.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_552 - Optimizing 3D Bioprinting: A comprehensive approach to bionk performance

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Introduction/Objectives
Bioprinting is emerging as a transformative tool in biotechnology, with applications ranging from cancer research and drug testing to scaffold fabrication. However, as the technology becomes more prevalent, several challenges have arisen. A key issue is the lack of standardized protocols, as many techniques are tailored to specific bioinks. This highlights the pressing need for Bioprinting standardization. One critical but often overlooked factor in filament quality analysis is cell viability. Elevated pressure values can compromise cell viability due to increased shear stress within certain regions of the nozzle. Conversely, lower cell-safe pressure values may lead to irregular filament formation or nozzle clogging. Optimizing this fundamental Bioprinting step is essential for effective bioink characterization and for improving future constructs. Thus, the primary objective of this work is to establish a Bioprinting protocol that enhances bioink printing performance. Furthermore, we used an original photo-curable bioink of interest in our lab, which consists of gelatin methacryloyl and egg white proteins, to carry out our primary objective.

Methods
Evaluating printing quality involves three essential tests: extrusion, deposition and printability. The process begins with the extrusion test, which examinates the relationship between printing pressure and the printing deposition rate, identifying the optimal pressure for consistent extrusion. Following this, deposition test analyzes the filament quality deposited under different speed and pressure conditions. Finally, printability test evaluates structural accuracy. To facilitate this evaluation, a 3D-printed lens support is created for precise image capture, and a custom Python script is developed to measure printing quality. Finally, Finite Element analysis is performed to link pressure values with shear stress and fluid flow dynamics, offering a comprehensive understanding of bioink behavior under varying printing conditions.

Results
Optimal filament quality is achieved by carefully balancing printing pressure and speed. The results demonstrate that higher printing pressure increase the volume of bioink deposited, while higher speeds reduce it. Systematic fine-tuning of the printing parameters ensures consistent and reliable filament deposition.

Secondary analysis revealed additional challenges, including tip clogging, filament dragging, and filament collapse.

Given the non-Newtonian behavior of the bioinks, a more detailed analysis is conducted. Finite Element fluid simulations further supported these findings, highlighting the correlation between fluid velocity, shear stress and nozzle geometry.

Conclusions
The proposed approach streamlines the 3D Bioprinting process by minimizing material waste and offering a systematic method for identifying optimal printing parameters. By integrating both quantitative and qualitative analysis, it provides a comprehensive framework for evaluating printing quality. This foundation paves the way for advancements in 3D Bioprinting, enabling more consistent and precise applications in tissue engineering.

Topic: Bioprinting & Biofabrication
Subtopic: Bioinks
Type: Oral presentation

TERMIS25_638 - Ultra-small 3D Bioprinting of Hyaluronic acid Hydrogel: A Platform for Studying Cell Interactions

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Introduction/Objectives
3D bioprinting is an innovative technology bridging tissue engineering and additive manufacturing, enabling the precise, layer-by-layer deposition of bioinks to fabricate implantable tissues and advanced biological models. Despite notable progress, developing an ideal bioink with balanced biological, physical, and mechanical properties remains a challenge. Hyaluronic acid (HA), a key extracellular matrix component, shows promise as a bioink due to its biocompatibility. However, its poor mechanical strength and rapid degradation necessitate crosslinking. This study aims to develop a shear-thinning, dynamic, disulfide-crosslinked HA hydrogel that provides a stable matrix for advanced 3D bioprinting applications.

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Methods
To preserve HA's intrinsic properties while enhancing its functionality, we modified less than 10% of the HA backbone with cysteine groups, forming a dynamic, disulfide-crosslinked hydrogel via pH adjustment. While disulfide-crosslinked hydrogels often suffer from slow gelation kinetics at physiological pH, limiting their usability in 3D bioprinting, we addressed this challenge by employing a novel catalyst. This significantly reduced the gelation time, enabling practical use in bioprinting and also it can function as a radical scavenger. The optimized hydrogel was evaluated for its ability to encapsulate cells and support complex 3D bioprinting with fine needles (32G, 108 μm inner diameter).

Results
The disulfide-crosslinked HA hydrogel successfully encapsulated human mesenchymal stem cells (hMSCs) and chondrocytes, maintaining their viability and enabling interaction within the matrix. The hydrogel exhibited a dual-degradation mechanism: enzymatic degradation by hyaluronidase targeting the HA backbone and reductive degradation by glutathione, which is secreted by cells and targets the disulfide crosslinks. This dual mechanism allowed us to control the degradation rate based on the cellular environment and the density of encapsulated cells. Using a 32G needle, we achieved high-resolution, layer-by-layer 3D bioprinting of intricate structures. Co-cultures of hMSCs and chondrocytes within the printed constructs facilitated the investigation of cell-cell interactions, migration, and the dynamic behavior of cells in a 3D environment. Additionally, we demonstrated the hydrogel's utility in studying the modulatory effects of hMSCs on inflammation-stimulated chondrocytes within a controlled microenvironment, showcasing its potential for in vitro modeling and regenerative applications.

Conclusions
Cysteine-modified HA hydrogels offer a promising solution for advanced 3D bioprinting, especially for high-precision applications using ultra-fine needles. The ability to achieve gelation with a single compound, without the need for mixing or complex crosslinking procedures, enhances the system's practicality and consistency. This hydrogel platform represents a significant step toward creating sophisticated in vitro models for tissue engineering and regenerative medicine.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_718 - Advanced solvent-free ink formulation for nano-resolution polymer-bioceramic DLP printing toward bone regeneration applications

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Introduction/Objectives
The development of advanced polymer precursor ink formulation for incorporating ceramics is critical for fabricating high-resolution polymer-ceramic scaffolds that replicate the structural and functional properties of natural bone. However, existing ink precursors pose significant challenges, including limited ceramic loading, low nanoscale precision, poor biocompatibility, non-removable, high viscosity, and short shelf life. Additionally, solvent-based methods often pose environmental and handling concerns while hindering scalability and storage efficiency. This study aims to address the stated limitations by creating a solvent-free, easily removable, reusable ink precursor for polymer-bioceramic DLP 3D printing. The developed ink formulation aims to provide enhanced stability, printability, and osteogenic potential, advancing the fabrication of bone tissue engineering scaffolds for clinical applications.

Methods
A solvent-free ink precursor was developed for incorporating bioceramic nanoparticles. The ceramic incorporation is done by a novel rapid mixing strategy at room temperature. Additionally, the bioceramic phase is functionalized in a way such that it does not precipitate in the polymer phase even with high loading (≥50 wt.%), ensuring a homogeneous dispersion. The rheological properties of the ink are optimized to enable nano-resolution printing in DLP systems. The polymer-bioceramic scaffolds were then tested for their physicochemical, mechanical properties, bioactivity (via simulated body fluid testing), and cellular response using osteoblast cells for adhesion, proliferation, and osteogenic differentiation.

Results
The optimized polymer-ceramic ink demonstrated excellent printability in nanoscale DLP systems. The nanoscale printing capability was validated by fabricating scaffolds with intricate geometries and pore sizes having 200 nm resolution. FESEM and Micro-CT data ensured the microstructure, porosity, and distribution of ceramic particles in the polymer matrix. The ink was found to be water or ethanol-soluble, facilitating easy handling and removal from containers, unlike commercial resins. The ink is found to be reusable multiple times with a shelf life of 3 months. Rheological properties ensured low viscosity ink even with high ceramic loading to enable precision printing. Excess of photoinitiator causing toxicity is easily removable from the printed scaffold by repeated washing. The scaffold confirmed significant bioactivity due to apatite layer formation after immersion in simulated body fluid. Cell material interaction displayed significant osteoblast cell attachment, proliferation, and elevated alkaline phosphatase activity, indicating osteogenic differentiation.

Conclusions
The developed polymer ink precursor and advanced DLP printing methodologies successfully addressed key challenges in polymer-bioceramic scaffold fabrication, including nanoscale precision, and biocompatibility. The solvent-free composition, made with commonly available chemicals, holds significant potential for scalability. This approach paves the way for fabricating customized, functional bone implants for clinical use. Future work will focus on scaling up the process and conducting in vivo studies to validate its translational efficacy.

Topic: Bioprinting & Biofabrication
Subtopic: Bioinks
Type: Oral presentation

TERMIS25_842 - Formulation of a methacrylated collagen bioink with controlled functionalization for corneal 3D bioprinting

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Introduction/Objectives
Corneal transplantation is one of the main treatments for corneal damage, a leading cause of visual impairment worldwide. However, it is currently limited by several factors, primarily the shortage of donor corneas. This shortage has driven the development of bioartificial corneal substitutes, one promising approach being three-dimensional (3D) bioprinting, which allows the production of complex, cell-loaded constructs. Within this technology, digital light processing (DLP) is a very promising approach for creating corneal constructs, as it allows a higher printing resolution compared to extrusion bioprinting, allowing to better replicate the smooth

curvature of the native cornea. This technology also allows to bioprint the convex geometry of the tissue without using support structures that are necessary for extrusion bioprinting.

Recent advances in cornea 3D bioprinting have highlighted various collagen-based bioinks, particularly methacrylated collagen (ColMA) for digital DLP bioprinting. However, a standardized methacrylation protocol for collagen has yet to be established, with existing studies reporting varying degrees of functionalization (DoF) and often employing lengthy and labor-intensive reaction protocols. Moreover, certain methodologies may compromise the native triple helix structure of collagen, a critical determinant in the cell-matrix interaction. The potential for protein denaturation raises concerns that some studies may have inadvertently synthesized methacrylated gelatin (GelMA) instead of ColMA. GelMA, while related, exhibits distinct mechanical, biochemical, and cellular interaction properties compared to ColMA. This underscores the need for precise reaction conditions to ensure the integrity of the collagen's native structure during methacrylation. In this work, we propose a collagen methacrylation reaction optimized to reliably obtain ColMA of desirable DoF, while at the same time preserving the protein structure.

Methods
The methacrylation reaction was studied by modifying several variables such as pH, time, and concentrations. The DoF was quantified through nuclear magnetic resonance (NMR), and the protein structure was evaluated through circular dichroism (CD). The obtained ColMA was then used to formulate a DLP-compatible bioink that was analyzed for viscosity and bioprintability. The transparency and rheological properties of the printed hydrogels were analyzed with a spectrophotometer and a torsional rheometer, respectively. Human corneal stromal cells (hCSK) were embedded in the bioink to analyze cell viability and metabolic activity through Live/Dead and AlamarBlue assays.

Results
The reaction was optimized across various parameters, consistently yielding ColMA that retained its triple helix structure. ColMA with desirable DoF were selected to formulate the bioink, which demonstrated suitable properties for DLP bioprinting. The resulting hydrogels exhibited favorable rheological properties and transparency within the visible light spectrum, along with high cell viability and metabolic activity.

Conclusions
In this work, we have optimized the reaction conditions to reliably obtain ColMA with specific DoF that shows adequate properties for DLP bioprinting as well as promising results for its use in cornea applications, allowing to create constructs with potential use for patient transplantation, *ex vivo* experimentation and pharmacological studies.

Topic: Bioprinting & Biofabrication
Subtopic: Bioinks
Type: Oral presentation

TERMIS25_1040 - Designing Hyaluronic acid Hydrogel Crosslinking Chemistry for 3D Bioprinting

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Introduction/Objectives
This study aims to develop a versatile HA-based hydrogel bioink with dynamic disulfide and thiazolidine crosslinking to address the mechanical weakness and rapid degradation of conventional HA hydrogels. By combining shear-thinning behavior for printability with reversible and self-healing properties for stability, this approach provides a tunable matrix with enhanced mechanical properties, crosslinking kinetics, and cytocompatibility for advanced 3D bioprinting applications.

Methods
To design HA-hydrogels HA-cysteine and HA-aldehyde were synthesized following our previously established protocols (Adv. Funct. Mater., 2024, 34, 2307040). The HA-aldehyde was dissolved in phosphate-buffered saline (PBS, pH 7.4), while HA-cysteine was dissolved in degassed PBS (pH 5.0). Both solutions were prepared to a final solid content of 2%. The ratio of HA-cysteine to HA-aldehyde was varied (100:0, 75:25, and 50:50) to produce hydrogels with different crosslinking mechanisms: disulfide only, a combination of disulfide and thiazolidine, or thiazolidine alone.

After complete dissolution, the pH of the HA-cysteine solution was adjusted to 7.4 using NaOH. Human mesenchymal stem cells (hMSCs) were then incorporated into the HA-cysteine solution. The two solutions were thoroughly mixed for 10 minutes to initiate hydrogel formation. The resulting hydrogel was immediately loaded into a 3D printing syringe fitted with a 25G needle and assessed for cell viability.

Results
The dual-crosslinked HA hydrogel successfully encapsulated human mesenchymal stem cells (hMSCs), preserving their viability and promoting interaction within the matrix. This hydrogel demonstrated both enzymatic degradation by hyaluronidase as well as reductive degradation by glutathione secreted by the encapsulated cells, which cleaves disulfide crosslinks. These complementary degradation pathways allowed precise control of hydrogel degradation, tailored to the cellular environment and the encapsulated cell density. The rapid kinetics of the thiazolidine reaction significantly enhanced the initial viscosity of the hydrogel matrix, facilitating extrusion-based bioprinting, while the disulfide crosslinks provided structural stability and increased stiffness post-printing. Notably, cell viability studies revealed that the hydrogel effectively protected hMSCs from anoikis during extrusion, offering superior cell protection compared to extrusion in culture media alone.

Conclusions
This work highlights the potential of this hydrogel system to serve as a versatile bioink with tunable mechanical and degradation properties. By combining biocompatibility, structural robustness, and responsiveness to the cellular environment, this strategy paves the way for more advanced and effective 3D bioprinting techniques in tissue engineering applications.

Topic: Bioprinting & Biofabrication
Subtopic: Bioinks
Type: Oral presentation

TERMIS25_1211 - Development and maturation of a 3D bioprinted scaffold for tendon regeneration using pressure-based extrusion and stem cells

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Introduction/Objectives
One of the major challenges in 3D bioprinting is to obtain a biomechanical competent scaffold that can withstand high loads as it is required for tendon regeneration. In this study we propose a novel protocol for pressure-based extrusion bioprinting to create 3D scaffolds using a collagen bioink reinforced with collagen fibers, aiming to obtain superior mechanical properties. The ECM mimicking

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composition and anisotropy generated by the fibers is also expected to boost tenogenic differentiation of stem cells extracted either from tendon or adipose tissue samples. Overall, the proposed approach should allow researchers to generate a structurally stable, biocompatible, and functional 3D bioprinted construct that promotes cellular growth, differentiation, and extracellular matrix (ECM) production.

Methods
Stem cells were obtained from tendon and adipose tissues samples, under protocols previously established with St Antonio University Hospital, Porto. The cells underwent primary culture expansion before being embedded into a bioink consisting of a commercial collagen hydrogel reinforced with collagen fibers. This bioink was then extruded using the CellInk BIO X bioprinter, with optimization of bioprinting parameters such as nozzle pressure and diameter, extrusion rate, and temperature. Scaffold characterization included a Live/Dead assay to measure cell viability immediately post-bioprinting and over a 21-day culture period. Scanning Electron Microscopy (SEM) and confocal microscopy were employed to assess the scaffold's microstructure and alignment of collagen fibers. Immunofluorescence staining provided spatial information on protein localization within the scaffold. Gene and protein expression were analyzed through quantitative real-time PCR (qRT-PCR), and Western Blotting (WB), respectively, to evaluate the differentiation of stem cells towards a tenogenic phenotype.

Results
Primary stem cells isolated from tendon and adipose tissues were successfully cultured and incorporated into a collagen-functionalized hydrogel, forming a stable bioink. Post-bioprinting Live/Dead assays showed a cell viability rate of 65%, which increased to over 70% by day 7, indicating high compatibility with the bioprinting process. SEM analysis displayed a well-organized scaffold with collagen fibers aligning alongside cells, mimicking tendon structure. Immunofluorescence staining identified key extracellular matrix proteins such as collagen I, collagen III, and tenomodulin, suggesting active ECM production. qRT-PCR and Western Blot results confirmed the upregulation of tenogenic markers, including SCX-A, COL1A1, and TNMD, indicating early differentiation of stem cells towards a tendon phenotype.

Conclusions
This study proposes a standardized bioprinting workflow for creating tendon-mimetic 3D scaffolds using primary stem cells and functionalized hydrogels with superior biomechanical performance. The anticipated results should provide a foundation for future tendon regeneration research and offer a protocol adaptable to other tissue engineering applications. The established workflow will allow researchers to explore new therapeutic strategies, test potential drugs, and optimize tissue-specific scaffolds using various cell types and biomaterial functionalization.

Topic: Bioprinting & Biofabrication
Subtopic: Bioinks
Type: Oral presentation

TERMIS25_1215 - Microfluidic Nanoprecipitation of BMP-2-Loaded Polymeric Nanoparticles Stimulating Osteogenic Differentiation in GelMA Scaffolds Bioprinted with Human Tendon-Derived Stem Cells

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Introduction/Objectives
This study presents a groundbreaking approach to bone regeneration, leveraging microfluidics-assisted nanoprecipitation to engineer polymeric nanoparticles (NPs) loaded with Bone Morphogenetic Protein-2 (BMP-2) for enhanced osteogenic differentiation. These precisely tuned NPs were integrated into GelMA (gelatin methacrylate) scaffolds through advanced 3D bioprinting techniques and combined with human Tendon Stem Progenitor Cells (hTSPCs). The resulting scaffolds were dynamically cultured in a perfusion bioreactor over 21 days, recreating a biomimetic microenvironment tailored for cutting-edge tissue engineering applications.

Methods
PLGA (poly(lactic-co-glycolic acid))-based nanoparticles were synthesized via a nanoprecipitation technique assisted by microfluidics, achieving precise control over size distribution (DLS: dynamic light scattering) and BMP-2 release kinetics (ELISA). These nanoparticles were incorporated into GelMA bioinks for scaffold bioprinting (Dr. INVIVO 4D2, ROKIT Healthcare). hTSPCs were expanded and encapsulated in the bioink to create cell-laden scaffolds. Dynamic culture was performed using a perfusion bioreactor, with periodic assessments for cell viability (Live/Dead assay), osteogenic differentiation (RT-PCR for gene expression, histological analysis with Alizarin Red, immunofluorescence, and Western blot for protein markers), and scaffold integrity.

Results
Comprehensive characterization confirmed the uniformity of the nanoparticles (particle size: 124.7nm, PDI < 0.2) and their precise and sustained release of BMP-2 throughout the culture period. Cell viability assays highlighted the compatibility of the bioprinting process and scaffold environment, ensuring robust survival of hTSPCs. Osteogenic differentiation was powerfully demonstrated by the upregulation of key osteogenic markers (e.g., *BGLAP*, *SPP1*, and *ALP*), the deposition of a calcium-rich extracellular matrix visualized via Alizarin Red staining, and heightened BMP-2-associated protein production confirmed through Western blot analysis. Histological and molecular evaluations further validated the seamless integration of the scaffold with the embedded cells, showcasing its potential for advanced bone tissue engineering.

Conclusions
Integrating BMP-2-loaded nanoparticles into GelMA scaffolds, achieved through cutting-edge microfluidics and bioprinting techniques, effectively drives osteogenic differentiation in hTSPCs. This innovative system is a robust bone tissue engineering and regenerative medicine platform, seamlessly combining precise drug delivery, biomimetic scaffold architecture, and dynamic culture conditions. Future efforts will focus on refining nanoparticle payloads and scaffold configurations to maximize clinical translation and unlock its full therapeutic potential.

Topic: Bioprinting & Biofabrication
Subtopic: Bioinks
Type: Oral presentation

TERMIS25_1257 - ATP/collagen coacervates as new precursors for bioprinted dense collagen matrices

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Introduction/Objectives
Type I collagen, a key component of the extracellular matrix, has garnered significant attention as a regenerative biomaterial. Biocompatible and low-immunogenic, collagen provides mechanical support and biological cues making it ideal for fabricating biomimetic scaffolds. *In vivo* it presents a hierarchical organization in which collagen triple helices self-assemble into highly ordered fibrils in a process called fibrillogenesis. *In vitro* this process can be triggered, by changes in pH and temperature, dependent on the collagen concentration.

Integrating collagen's intrinsic ability to self-assemble with a bottom-up fabrication technique such as bioprinting could bridge the gap in recreating biological tissue analogues. However, printing complex structures using collagen in its native form is hindered by

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use at sub-physiological concentrations. Moreover, the dramatic viscosity increase with concentration, makes 3D printing collagen solutions in the range of concentrations required for tissue modelling, a difficult task.

Here, we report a new strategy for formulating highly concentrated collagen bioinks with drastically reduced viscosity able to bypass current limitations in 3D bioprinting type I collagen.

Methods
Using adenosine triphosphate (ATP) we induce a liquid-liquid phase separation where collagen molecules aggregate and adopt a stacked rod-like packing in the form of Segment Long Spacing (SLS). By simple coacervation this suspension of concentrated coacervates can be adjusted to achieve a high concentration.

The resulting bioink was characterized by rheological measurements to assess the impact on printability. Microscopic analysis was used to investigate collagen distribution and organisation in the printed constructs. The formulation and the rheological properties of the bioink were correlated to the viability, proliferative status and morphology of Normal Human Dermal Fibroblasts in a dense collagen matrix.

Results
Induced by ATP, collagen assumes a macromolecular architecture characterized by rod-like bundles of perfectly aligned collagen heterotrimers of approximately 300 nm with no repetitive pattern.

Rheological analysis revealed a ten-fold decrease in dynamic viscosity with respect to collagen in solution at equivalent concentration, giving rise to printable suspensions. Microscopic analysis pointed at a different fibril organisation compared with the classical formulation, giving rise to an architecture prone for cell colonization. Importantly, coacervation doesn't impair fibril formation and supports ordered collagen motifs formation.

The biocompatibility was assessed by measuring fibroblasts metabolic activity in a dense collagen matrix (60 mg/mL), showing that the composition of the bioink doesn't interfere with cellular processes in the studied timescale. Moreover, as seen by confocal microscopy fibroblasts display physiological characteristics and successfully manage to colonize and remodel the matrix.

Conclusions
Our approach allows the formulation of an innovative collagen bioink, with drastically reduced viscosity, compatible with extrusion-based bioprinting at physiologically relevant concentrations. Relying solely on collagen self-assembly to mimic structural tissue features, we ensure the fabrication of biocompatible and biomimetic constructs, that have native-like architecture, improved mechanical properties and support cellular processes.

Topic: Submit to SYMPOSIUM
Subtopic: Biomaterials design strategies for tissue engineering
Type: Oral presentation

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TERMIS25_233 - PVA-based hydrogels for skin regeneration: Using MMP-Sensitive crosslinkers for cellular remodeling

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Introduction/Objectives
This study aims to develop a tissue-responsive hydrogel scaffold to support skin tissue regeneration by comparing different crosslinkers, including a matrix-metalloproteinase (MMP)-sensitive crosslinker which facilitates cell-matrix remodeling. The different materials produced were characterized in an acellular and cellular context, aiming to enhance cell viability and proliferation in tissue regeneration applications.

Methods
Norbornene-functionalized polyvinyl alcohol (PVA-NB) was used as the hydrogel precursor and was rapidly crosslinked under UV-light (<60 s) with cell-cleavable and non-cleavable crosslinkers, such as an MMP sequence flanked by two cysteines, and a dithiolated Polyethylene glycol (HS-PEG-SH), respectively, in presence of 0.1 wt% of Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). The final formulation concentration of PVA-NB was 5 wt%, and 50% of the norbornene groups were used for the crosslinking. A thiolated cell-adhesion promoting peptide sequence including the Arginine-Glycine-Aspartic Acid (RGD) motif was also incorporated in the hydrogels. The produced matrices were characterized in terms of swelling, long-term stability, degradation kinetics and photorheology. After all conditions were studied and optimized, fibroblasts were encapsulated within the matrix and the viability, adhesion and cell-matrix interactions were evaluated.

Results
A dithiolated PEG with a similar molecular weight to the MMP was used to better understand and compare the effects inflicted upon the material by the different crosslinkers. The two different hydrogels exhibited similar photorheological behavior, such as reactivity and storage modulus of approximately 2 kPa. Their swelling behavior was also very similar as well as their stability over time under physiological conditions, where the hydrogels were not dissolved after 45 days. However, a different trend was observed after investigation of their accelerated enzymatic degradation kinetics using collagenase. In fact, the PVA-MMP hydrogels had degraded within 24 hours whereas the non-cleavable PVA-PEG hydrogels were only 20% degraded after 5 days. Immortalized adult dermal fibroblasts were mixed in the formulation to enable observation of the cell-matrix interactions. The viability remained high after 14 days, proving that the matrices exhibit high biocompatibility. Further microscopy observation after 2 weeks in culture revealed that the cells had longer protrusions and a diversity of morphologies in the PVA-MMP hydrogel, whereas cells remained rounded when encapsulated in the PVA-PEG hydrogels, despite the presence of RGD in both formulations.

Conclusions
The cell-responsive hydrogel platform developed in this study provides a dynamic environment for tissue regeneration. This cleavable design is well-suited for clinical applications, as it provides mechanical integrity and the capacity for cellular remodeling. Furthermore, the material's adaptability to numerous formats, including electrospinning, granular hydrogels, light-based high-resolution techniques (Two-photon polymerization or volumetric printing) and injectable hydrogels expands its potential utility in future regenerative therapies.

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Topic: Submit to SYMPOSIUM
Subtopic: Biomaterials design strategies for tissue engineering
Type: Oral presentation

TERMIS25_651 - Composite bioink of ADA-GEL-short ribbon fillers for skeletal muscle tissue bioprinting

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Introduction/Objectives
Skeletal muscle tissue has a medium self-repairing potential; however, damages beyond its self-repair capacity can require immediate transplantation. Bioengineered skeletal muscle aims to mimic the anisotropic and organized structure of skeletal muscle with its densely packed aligned myofibers. Such engineered tissue can generate similarly high contraction forces in vitro beneficial for

biohybrid robot applications and can be used for regeneration of damaged tissues such as volume muscle loss or as cellular agriculture and engineered meat. To achieve the cellular alignment and hierarchical structure of muscle tissues in bioprinted constructs, we present a composite bioink based on the oxidized alginate-gelatin (ADA-GEL) containing ribbon-shaped fillers as cell carriers and investigated the properties of this bioink under various cross-linking conditions and its 3D bioprinting.

Methods
C2C12 myoblast cells were immobilized on 100 μm microfabricated poly(lactic-co-glycolic acid) (75:25 PLGA) ribbons, which were embedded in an oxidized alginate-gelatin hydrogel matrix (2.5% ADA - 3.75% GEL). To enhance cell adhesion, PLGA ribbons were functionalized with fibronectin using plasma treatments ranging from 30 to 50 W for 2 to 10 minutes. Coating quality was evaluated through X-ray photoelectron Spectroscopy (XPS), fluorescence microscopy, contact angle measurements, and scanning electron microscopy (SEM). The hydrogel composite was cross-linked through both enzymatic and ionic crosslinking, using 2.5% transglutaminase (mTG) and varying $CaCl_2$ concentrations (20, 40, 60 and 100 mM). The resulting hydrogels were assessed for flow properties, compression strength, pore size (via cryofixation and lyophilization), biodegradation, and cell adhesion, proliferation and myogenesis. The 3D printing of the bioink with ribbon-shaped filler was explored and its effect on cell alignment.

Results
Pore area analysis, along with degradation and mechanical testing, identified 60 mM $CaCl_2$ as the optimal concentration for crosslinking of ADA-GEL. This gel achieved a compression modulus of 11.5 kPa, comparable to native skeletal muscle tissue with a high cell viability of 84% and high proliferation over 7 days. Rheological characterization of the ADA-GEL composite hydrogel with 0.1% ribbon-shaped fillers confirmed its suitability as a bioink, displaying ideal shear-thinning and flow properties. The 30 W oxygen plasma treatment of PLGA ribbons, for 5 minutes, provided optimal conditions for fibronectin immobilization and cell adhesion and spreading, as demonstrated by XPS, SEM, water contact angle measurements, and cell adhesion assays. After differentiation of the myoblasts on ribbons and embedded in ADA-GEL, the alpha actinin marker was detected in cells after 5 days of differentiation. High alignment of the ribbon fillers was achieved after optimization of the printing parameters.

Conclusions
These findings underscore the potential of the 2.5% ADA - 3.75% GEL composite bioink with ribbon fibers for developing anisotropic tissue models. Our study revealed that the commonly used $CaCl_2$ crosslinker concentration of 100 mM caused cell toxicity, likely due to the role of Ca^{2+} as a signaling molecule in muscle cells. Bioprinting cell-laden fillers enable cellular alignment within 3D structures, supporting myogenesis and the formation of functional muscle microtissue.

Topic: Submit to SYMPOSIUM
Subtopic: Biomaterials design strategies for tissue engineering
Type: Oral presentation

TERMIS25_941 - Topographically-textured microparticles as a modular platform for mechanically-guided cell patterning in bottom-up tissue engineering

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Introduction: The instructive capacity of physical microenvironments in directing cell fate represents a fundamental yet underexplored biological mechanism. Mesenchymal stem cell (MSCs) engineering is advancing rapidly with novel biomaterials that modulate cell fate via mechanobiological cues. Topographically-textured microparticles can be leveraged as modular platforms for modelling stem cell and tumour niches via precise control over 3D physical features. This offers transformative potential in bone regeneration and disease modelling.

Methods: Our approach employs polymeric microparticles engineered with varied surface topographies via controlled phase-separation within polylactic acid matrices[1]. We have employed a design-of-experiments (DoE) approach to fine-tune microparticles' surface features, advancing our understanding of cell signalling in response to precisely engineered microenvironments. RNA-sequencing was also used to study the transcriptomic profiles of MSCs cultured on different microparticles designs.
Results: Our studies demonstrated that precisely designed poly(D, L-lactide) microparticles with controlled surface architectures influence MSCs attachment, proliferation, and induce osteogenic responses independent of biochemical additives[1]. Subsequent mechanistic investigations revealed this process occurrs through activation of canonical Hedgehog signalling[2]. The temporal sequence revealed by transcriptomics analysis has uncovered that these 3D-topographical cues initiate a coordinated developmental patterning program. The expression of key mechano-sensing elements, such as *ITGB4* and *PIEZO2*, were significantly upregulated, driving developmental patterning responses modulated by transcriptional regulators such as *PAX7* and *HAND2,* as well as significant upregulation of Hedgehog signalling pathway components, including *SMO*, *PTCH1* and *GLI1*. This underlines a novel mechanistic pathway of mechanically-guided MSCs response, expanding current paradigms of cell fate control. We have also methodically optimised surface-design parameters using a DoE strategy, enabling practical applications in tissue engineering and fundamental mechanobiology research.
Conclusions: Our findings underscore the versatility of topographically-engineered microparticles in creating cell-instructive 3D microenvironments. A systematic investigation of engineered topographical features revealed a sophisticated program of mechanically-guided developmental patterning distinct from traditional differentiation. This opens avenues for next-generation regenerative therapies, high-throughput drug screening, and bone disease modelling by leveraging the inherent mechano-sensitivity and signalling plasticity of MSCs.
Funding: Academy of Medical Sciences Springboard Award [SBF008\1057]
1 Amer *et al*, Biomaterials, 2021
2 Ghuloum *et al*, Biomaterials Adv, 2023

Topic: Submit to SYMPOSIUM
Subtopic: Biomaterials design strategies for tissue engineering
Type: Oral presentation

TERMIS25_1010 - Freeform 3D Printing of Customized Cell-adsorptive cryogels

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Introduction/Objectives
In tissue engineering (TE), hydrogels have proven to be valuable platforms for 3D cell culture by mimicking the extracellular matrix. However, they face significant challenges, including poor nutrient and oxygen diffusion to their core, which leads to reduced cell viability and densities. Furthermore, their injectability is limited under higher shear stress conditions due to irreversible structural damage.

To address these issues, we present an innovative approach using 3D printing cryogels for on-demand cell absorption and their injection of cell-loaded complex structures. The intricate porous network of cryogels enhances diffusivity, injectability, and shape-recovery properties, making them a promising tool for minimally invasive biomedical applications.

Methods
GelMA synthesis (insertion of methacryloyl groups), biomaterial ink formulation (determined through rheological assays), and printing conditions (pressure, speed, and temperature) were developed and optimized. The mechanical properties (evaluated using a rheometer and Instron) and biological assays of 3D printed structures were performed and compared with conventional PDMS casting cryogels.

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Our method enhances injectability and employs a single-component biomaterial ink, based on GelMA and visible light cross-linking, to fabricate 3D scaffolds.

Results
GelMA with a degree of substitution of 89 % and the 2 % (w/v) concentration was determined to be the most adequate for the ink, resorting to macroscopic and rheological data. The GelMA cryogels exhibit injectability, shape-recovery, and rapid hydration properties, capturing over 85% of cells and spheroids. We assessed the viability of human adipose-derived stem cells encapsulated on the scaffold over 14 days, considering nozzle shear stress during injectability.

Conclusions
Our results shed light into the relationship between pore content, injectability, and cell absorption capacity. The dried cryogels can be stored and used on-demand to absorb liquid suspensions and, if needed, rapidly encapsulate cells and cellular aggregates. GelMA 3D printed cryogels could be proposed as on-demand cellularized scaffolds for minimal invasive clinical application by simply resuspending them in a biopsy-isolated suspension of stem cells. The presented 3D printing approach offers customization, on-demand production, and improved mechanical properties, advancing the development of robust cryogels capable of withstanding high pressure while accommodating cells. This innovation has the potential to shape the future of TE, particularly for applications such as craniofacial defects, where different types of tissue with varying requirements are present, and minimally invasive techniques are essential due to the size and complexity of the injury.

Acknowledgements
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Topic: Submit to SYMPOSIUM
Subtopic: Bioinspired Approaches: Using Natural Polymers to Engineer the 3D Tumor Microenvironment.
Type: Oral presentation

TERMIS25_247 - Light gellan gum: a new mechanotuner for high-precision stiffness engineering in 3D microenvironments

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Introduction/Objectives
The complex mechanics of living tissues is a challenging yet fundamental property to consider in any biomaterial-based strategy for tissue engineering, both targeting healthy tissues or disease models, like cancer. Many of the more widely used bioactive materials, like collagen, reconstituted basement membranes, decellularized ECM gels, and others, present the critical issue of very low stiffness and limited mechanical tunability. More structural polymers, like the natural-derived Gellan Gum (GG), are interesting ionic-crosslinking reinforcements but typically limited due to their high molecular weight, viscosity, and inability to blend with other polymers. In this work, we aimed to combine the best of the two worlds by obtaining a novel Gellan Gum alternative and explore it to control and manipulate the mechanics of 3D microenvironments in a standardized way, independent of composition.

Methods
We have studied and for the first time explored low-molecular weight GG (light GG) for applications in cell encapsulation. By exposure to controlled gamma-radiation, we successfully tuned the molecular weight of GG down in incremental steps, down from around 1 MDa (native). Our results show that the treatment results in shorter polymeric chains, significantly less viscous solutions, which can be easily manipulated and virtually interpenetrate any other hydrogel network. We have then explored a range of applications for this new hydrogel material across different cellular scenarios, from static encapsulation of cartilage cells to adhesive collagen on stem cell-laden environments, and reconstituted basement membrane on cancer cell-laden environments.

Results
Our results demonstrate that light GG can be uniquely used as an orthogonal mechanical switch to reinforce hydrogel mechanics, across different orders of magnitude, without compromising the cellular interaction of more bioactive components. The unique structural stability of the polymer was capable of important feats like fully preventing collagen hydrogel contraction, as well as adjusting its mechanics to control the differentiation of cells. In cancer microenvironments, we've shown that light GG can broadly modulate the mechanics of reconstituted basement membrane (e.g. GelTrex) and yield radically different cancer responses and phenotypes within the same cell lines by precisely mimicking the different mechanics of tumor, pre-carcinogenic, or healthy tissues, with kilopascal precision.

Conclusions
Overall, light GG has the potential to be implemented as a mechanotuner in virtually any hydrogel-based approach, with very strong potential on the engineering of biomimetic 3D microenvironments. It can also be further combined with technologies such as 3D printing and biofabrication for added complexity.

Topic: Microscopy & Advanced Imaging
Subtopic: AFM, traction force
Type: Oral presentation

TERMIS25_664 - Enhancing Large-Scale Atomic Force Microscopy Mechanical Characterization with SmartMapping

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Introduction/Objectives
Atomic force microscopy (AFM) is crucial for nanoscale mechanical property mapping, offering high-resolution characterization of stiffness, adhesion, and viscoelasticity. This capability is essential for understanding material behaviour in complex structures such as living cells, tissues, and biomaterials, thereby driving forward studies of cell behaviour, disease progression, and drug treatments. Nevertheless, challenges such as sample roughness and limited lateral scanning range often hinder large-scale mechanical mapping, particularly for complex and heterogeneous specimens including biopolymers, cells spheroids/organoids, and tissues.

Methods
We have developed a new SmartMapping tool that addresses these challenges by coordinating AFM head motors and XYZ-piezo movement. This innovation enables continuous, high-resolution mapping over extensive areas without user intervention, enhancing the precision and efficiency of AFM.

Results
We successfully tested this feature for comprehensive analysis of hydrogels with different stiffnesses (1 kPa and 50 kPa), analysing centimetre-scale areas and creating detailed mechanical property maps. Additionally, we evaluated the applicability of the new feature for analysing 2D cell populations seeded on hydrogels and high grade serous Ovarian cancer cell spheroids exceeding 100 µm in height. Furthermore, we assessed 600 µm thick vibratome-sectioned neuroblastoma tumours with very low stiffness (50-100 Pa) embedded in low-melting agarose gels, demonstrating that despite their roughness, such tissue samples can be effectively analysed.

Conclusions
The new SmartMapping feature significantly advances AFM capabilities, enabling precise and efficient large-scale mechanical

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mapping. This development opens new avenues for studying a wide range of samples, from complex biopolymers to soft biological tissues, enhancing the scope and impact of automated AFM in biomedical and biomaterial research.

Topic: Stem Cells
Subtopic: Differentiation, migration, activation
Type: Oral presentation

TERMIS25_758 - Tuning vibrational parameters for mechanically induced osteogenic response

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Introduction/Objectives
Stem cell therapies are a rapidly growing area of research to treat a range of conditions, from cardiovascular diseases to skeletal diseases, such as osteoporosis [1]. Mesenchymal stem cells (MSCs) may be the most commonly used in clinical studies, with a large focus on developing cell therapies to treat bone diseases [2]. Inducing osteogenesis in MSCs may be achieved chemically, however a more attractive option harnesses the mechanosensitive nature of these cells, applying mechanical stimulation, such as vibration, to direct stem cell differentiation. Whilst there have been many studies using vibration to induce an osteogenic response in cells, few agree on the optimal vibration conditions to drive osteogenesis in MSCs [3]. Therefore, this study looked to utilize nanovibrational stimulation, which has successfully applied nano-meter amplitudes to direct MSC differentiation toward osteogenesis, and explored tuning the vibrational parameters to optimise osteogenic differentiation in MSCs [4].

Methods
MG63 cells were used in initial optimisation studies to identify the optimal vibration conditions to induce osteogenesis. Frequency (100 Hz – 10 kHz), amplitude (30 – 90 nm), directionality (parallel/perpendicular to the cell monolayer) and duration of vibration (4 hours/day or continuous) were all explored to identify the optimal parameters resulting in increased osteogenic expression. Morphology and protein expression were analysed using immunofluorescence, gene expression was assessed using quantitative polymerase chain reaction (qPCR), and mineralisation was investigated using Alizarin Red staining. Optimised vibration parameters were then applied to three MSC donors of different ages and genders, with the same analysis techniques used.

Results
Higher amplitudes resulted in increased late osteogenic marker expression, which agrees with previous nanovibrational stimulation studies [4]. However, cell response to different frequencies was mixed, with 10 kHz resulting in higher alkaline phosphatase (ALP) gene expression and osteocalcin (OCN) protein expression, whilst 1 kHz showed the highest mineralisation and osteonectin (ON) gene expression. For directionality, vibration parallel to the cell monolayer, whilst showing no increased osteogenic expression in MG63s, did increase nuclear gene expression of proteins known to be involved in the mechanosensitivity of the cell. Continuous stimulation showed increased expression of late osteogenic markers, compared to shorter applications of 4 hours/day. When applied to MSCs, some donors showed a higher osteogenic response to optimised vibration conditions, however donor response was mixed.

Conclusions
Here, optimal vibration conditions to induce osteogenesis were identified. A higher amplitude increased osteogenesis, and vibrating cells parallel to the cell monolayer may be increasing mechanosensitivity in cells. When optimised conditions were applied to MSCs, donors responded differently to vibration. This difference in response highlights the need for additional study into donor response to vibration in order to further optimise vibration conditions to induce osteogenesis for cell therapy use.

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Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_1130 - Matrix mechanics modulate cancer plasticity

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Introduction/Objectives
Metastasis is the primary cause of cancer-related morbidity and mortality, with phenotypic heterogeneity and plasticity playing critical roles in tumour progression and metastatic dissemination. The tumour microenvironment (TME) is a complex assembly of various cell types and extracellular matrices driving dynamic changes in cellular phenotypes and molecular states. Mimicking key biophysical and biochemical attributes of the TME in model systems offers a valuable platform for studying the mechanisms underlying cancer plasticity and progression. This study aims to develop advanced in vitro models that replicate aspects of the TME to investigate the dynamic interactions between cancer cells and their microenvironment, particularly focusing on the effects of extracellular matrix (ECM) mechanics on cancer plasticity.

Methods
We employed 2D hydrogel micropatterning techniques and 3D bioprinted matrices to create spatially addressable substrates that coordinate substrate-cancer cell interactions in melanoma and breast cancer models. The 2D microtumouors were designed to mimic features of the TME, such as mechanical stiffness and spatial confinement, enabling precise control over cancer cell organisation. To extend this approach, we utilized mechanically tunable 3D bioengineered tumour matrices using 3D drop-on-demand bioprinting. These matrices allow for systematic variation of ECM stiffness and architecture, facilitating the study of cancer cell responses to diverse mechanical and biochemical cues. Cancer cell behaviours, spatial organisation, and dynamic phenotypic transitions, were characterised using immunofluorescence imaging. Drug resistance of cancer subpopulations in response to matrix mechanics was evaluated through standard-of-care chemotherapeutic treatments and cell viability assays.

Results
Our hydrogel micropatterning approach demonstrated that confinement and stiffness could influence cancer cell partitioning, replicating organisational patterns observed in vivo. When applied to 3D bioengineered matrices, we observed that ECM stiffness and architecture differentially regulated cancer plasticity across various cancer models. Strikingly, softer matrices promoted a specific stem cell population with higher drug resistance and invasive phenotypes compared to stiffer matrices. The mechanically tunable matrices allowed us to replicate the intricate interactions between mechanics and plasticity observed in traditional 2D studies, while providing a more physiologically relevant context. Additionally, the ability to spatially control ECM properties revealed previously uncharacterised dynamics of phenotypic transitions, offering insights into how tumour growth and invasion processes are regulated by the TME.

Conclusions
Our models show that matrix stiffness and architecture differentially regulate plasticity in different cancer models. Our 3D matrices allow

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for precise and modular control of ECM properties and replicate the intricate mechanics-plasticity interactions observed in 2D.

The fabrication of multiple aspects of the TME allows better control over features responsible for progression, thereby providing improved tools for fundamental studies and drug development targeting specific cancer cell subpopulations.

Topic: Biomechanics & Mechanotransduction
Subtopic: Mechanotransduction
Type: Oral presentation

TERMIS25_1301 - Mechanotransduction of αβ T-cell receptor via remote magnetic field application can modulate human T-cell activation in-vitro

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Introduction/Objectives
Anti-cancer adoptive cell immunotherapies have recently achieved a major boost as promising treatment modality. Recruitment of patient's own T-cells to target and produce an immune response in tumour microenvironment requires activation and differentiation of naïve T-cells mediated by T-cell receptor (TCR) complex. Recent research in T-cell mechanobiology has established that TCR acts as an anisotropic mechanosensor, which can convert mechanical forces into biochemical cues required for activation, differentiation, and proliferation, upon signalling by mechanosensitive ion-channels, in a process called mechanotransduction. We demonstrate a novel immunoengineering strategy of TCR mechanotransduction called Magnetic Ion Channel Activation (MICA) by application of an external magnetic field onto magnetic artificial antigen presenting cell (aAPC) tagged T-cells.

Methods
Magnetic core-shell particles in the size range 250 nm – 2 μm with a superparamagnetic magnetite core were surface functionalized with anti-CD3ε and anti-CD28 monoclonal antibodies (mAbs) using simple 2-step carbodiimide coupling to the carboxyl groups, to function as aAPCs. Naïve $CD4^+$ and $CD8^+$ T-cells were isolated by negative selection magnetic enrichment from human peripheral blood mononuclear cells (PBMCs) taken from patient blood and labelled with magnetic aAPCs. Labelled cells were then activated *in-vitro* upon application of an external oscillating magnetic field onto the aAPC-tagged cells for 1 to 4 hours which in-turn produced mechanical stresses on the TCR-CD3 complex resulting in an effector response via downstream signalling pathways. This effector response was then measured as an endpoint 16 hours after magnetic force application by immunophenotyping of surface activation markers including CD69, CD25, CD154, and HLA-DR (MHC-II) by flow-cytometry.

Results
Magnetic force application on aAPC labelled naïve $CD4^+$ and $CD8^+$ T-cells induced increased expression of cell surface activation markers compared to labelled cells without magnetic force application and unlabelled cells. The activation expression was also higher compared to commercially available CD3/CD28 Dynabeads for T-cell activation for the same times of activation. Directional functionalisation of mAbs on the surface of magnetic aAPCs using protein G as a binding base coating enhanced activation compared to non-oriented random distribution, due to increased availability of TCR binding sites of the mAbs on aAPC surface.

Conclusions
The easily scalable MICA platform can modulate T-cell activation by using magnetic aAPCs and provides an improved activation over currently available commercial technologies for adoptive T-cell immunotherapies.

Topic: Submit to SYMPOSIUM
Subtopic: Recent advances in articular cartilage engineering
Type: Oral presentation

TERMIS25_1610 - Development of a Nonlinear Regression-Based Model for Predicting Load and Mitigating Swelling Effects in Superficial Zone Cartilage Injury

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Introduction/Objectives: Post-traumatic osteoarthritis (PTOA) is a leading cause of disability worldwide, yet no causal therapy exists to date. Ex-vivo mechanical injury models of articular cartilage play a critical role in identifying the early degenerative and pro-inflammatory events linked to PTOA development. Many models exclude the superficial zone (SZ) despite its significant clinical relevance. This study aimed to establish a reliable ex vivo model for injurious compression of SZ cartilage discs, mitigating swelling and curling effects commonly observed in explant studies. Proof-of-principle experiments were conducted to evaluate changes in SZ cell viability, apoptosis, and proliferation.
Methods: SZ cartilage discs (4mm diameter) were harvested from the femoropatellar grooves of 2-3-year-old bovines. Disc thickness was measured after preparation and prior to injury, using an incubator-housed loading device. Thickness increase over time was recorded. Each disc was compressed to its original thickness. The resulting loads were plotted against the applied compression. Pearson correlation tests assessed relationships between initial and post-swelling thickness and the load required for flattening discs to their original thicknesses. Regression analyses using TableCurve2D yielded the best-fit regression-based equation and coefficients for calculating required loads for disc flattening. Mechanical injury was applied via a compressive strain of 65% at 100% strain rate. Cell viability (Calcein/PI co-staining, n=111-135), apoptosis (Annexin-V/PI co-staining, n=72-93), and proliferation (Click-It™ EdU-Staining, n= 18-22) were assessed in flattened (n=66-122) and non-flattened discs (n=72-135). Cell numbers across the full-thickness (250μm) and upper layers (44, 90μm) of SZ discs were quantified using ImageJ.
Results: Pearson correlation analyses revealed significant correlations between the disc thicknesses at days 0 (T_0) and 2 (T_2; correlation coefficient cc: -0.811, $p<0.001$) and between the increase in thickness from day 0 to 2 (T_2-T_0) and the load required for flattening a disc back to its original thickness (L_{T0}, cc: 0.651, $p<0.001$). Multiple regression analyses performed with TableCurve2D established the equation L_{T0} =1.59 +(-0.59 *(T_2 -(-75.97 +3.16 *T_2^0.5 *LN(T_2)))) for calculating the load required for flattening a disc with thickness T_2. Flattening alone did not cause significant changes in viability. Importantly, the flattening applied prior to injurious compression improved the effects of injury, particularly in the most superficial 44μm of SZ discs, with significant differences in cell viability and cell death (per cm^2) between flattened, injured discs vs. not flattened, injured discs ($p<0.001$). Apoptosis was significantly increased both 24 and 96 hours after injury and, surprisingly, proliferation was significantly increased both 48 and 96 hours after injury.
Conclusions: The innovative method introduced here enables injurious compression of SZ cartilage discs as an ex vivo model for studying early post-traumatic events leading to PTOA. By mitigating the swelling and curling effects commonly observed in explant studies, this method improves model reliability. This was achieved through the practical application of pre-injurious compression to re-flatten curled discs, guided by a nonlinear regression-based equation that calculates the required load based on measurable disc thickness prior to injury.

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Topic: Submit to SYMPOSIUM
Subtopic: Biomimetic and Bioinspired Materials for Regenerative Medicine
Type: Oral presentation

TERMIS25_204 - Bioprinting Gelatin Methacryloyl-Polydopamine Hydrogels: A Bioinspired Path to In Situ Biomineralization and Bone Regeneration

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Introduction/Objectives
Developing scaffolds that replicate the structure and function of natural bone is critical for effective tissue regeneration.[1]. This study investigates the potential of bioprinting cell-laden GelMA-PDA hydrogel scaffolds and leverage the adhesive, osteogenic, and mineral-binding properties of polydopamine (PDA) [2] to promote *in-situ* biomineralization, thereby creating a microenvironment favourable for bone repair.

Methods
GelMA-PDA bioinks were prepared by combining 7.5% (w/v) GelMA hydrogels [3] with 0.02% (w/v) PDA nanospheres, [4] synthesized via self-oxidative polymerization. These bioinks were benchmarked against 7.5% (w/v) GelMA hydrogels, which were characterized to evaluate rheological properties (strain sweep, frequency sweep, temperature sweep, viscosity-shear rate) and self-supporting ability vis collapse test. The GelMA-PDA bioink was then loaded with osteoblast-like Saos-2 cells at a concentration of 5×10^6 cells/mL and extrusion printed using a CELLINK BIO X 3D printer (BICO Co., Sweden). The bioink was subsequently stabilized by photocrosslinking with a UV lamp (390 s, 365 nm, 2.9 mW/cm^2, Spectroline Co., USA). Following bioprinting, the scaffolds underwent physicochemical and mechanical characterization, and cell culture studies were conducted to assess cellular viability, proliferation, and biomineralization potential.

Results
Compared to GelMA alone, the GelMA-PDA bioink demonstrated substantially enhanced printability, in terms of collapse resistance and strand integrity. Rheological analysis indicated that the GelMA-PDA bioinks possessed a higher complex viscosity under various frequencies and reduced viscosity under ascending shear rates, supporting their suitability for extrusion-based bioprinting. Compressive strength tests revealed a higher Young's modulus (1.8 times) and toughness (2.1 times) in GelMA-PDA scaffolds compared to GelMA alone. Absorption and degradation assays showed that GelMA-PDA scaffolds retained greater mass over time, suggesting reduced degradation rates. Saos-2 laden GelMA-PDA bioinks were assessed using alamarBlue and LIVE/DEAD staining on days 1 and 7, which confirmed high cell viability and proliferation. Acellular bioactivity in GelMA-PDA scaffolds beside *in-vitro* biomineralization after 21 days revealed an enhancement in the mineral deposition, indicating a favourable environment for bone tissue regeneration.

Conclusions
The incorporation of PDA nanospheres substantially enhanced the printability, mechanical robustness, and stability against degradation of GelMA hydrogel bioinks. Moreover, the biocompatibility of this scaffold with Saos-2 cells, and the facilitation of *in-situ* biomineralization positions GelMA-PDA as a promising candidate for bone tissue engineering.

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Topic: Tissue Engineering
Subtopic: Biomimetic, multiphasic, or smart models
Type: Oral presentation

TERMIS25_379 - Self-assembling human in vitro model for bone-tendon interfaces; synergizing collagen fibrillogenesis, biomimetic mineralization, and cellular embedding within physical constraints

Bregje de Wildt, Jess Snedeker[1 2]

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Introduction
The enthesis is the tissue transition connecting tendon to bone, distributing stresses to transfer loads between these tissues. High mechanical demands make the enthesis prone to inflammation induced remodeling (enthesitis) and tearing, with currently poor clinical outcomes. To drive treatment development, a better understanding of the enthesis' innate regenerative potential is needed. However, current knowledge on enthesis regeneration mainly originates from studies in rodents which limit translation towards humans. Although a few human *in vitro* enthesis models have recently been developed, the two-way interactions between the graded matrix and cells remain largely overlooked. To enable mechanistic study of enthesis related cell-matrix interactions *in vitro*, we developed a one-step methodology to embed human tenocytes within a bone-mimetic mineralized collagen hydrogel. Subsequently, we generated biphasic (bone–tendon) self-assembling constructs using a physically-constraint hydrogel culture platform.

Methods
To achieve mineralization of collagen type I hydrogels, the polymer-induced liquid-phase precursor mechanism was used, allowing for intrafibrillar collagen mineralization in supersaturated solutions using poly-aspartic acid (pAsp). A basic collagen mineralization premix containing NaOH, $CaCl_2$ (10 mM), and optionally pAsp (100 μg/ml) was prepared. When a slightly viscous hydrogel was desired to enable biphasic casting, 2 mg/ml hyaluronic acid was blended into the premix. Subsequently, acidic rat-tail collagen solution was mixed into the premix (1.5 mg/ml), after which a K_2HPO_4 solution (5 mM) was added to the hydrogel to induce mineralization. Non-mineralized hydrogels consisted of collagen with or without hyaluronic acid. For cell embedding, hydrogels were mixed with tenocytes (7.5 * 10^5 cells/ml) isolated from human biceps tendons. Cell-free hydrogels were evaluated for mineralization using scanning electron microscopy and Fourier transform infrared spectroscopy (FTIR). Cell-laden mineralized and non-mineralized hydrogels were cast in both ends of custom-made molds, divided by a silicon separator. After casting, separators were removed, posts were placed at both ends of the mold, and hydrogels were polymerized for 45 minutes at 37 °C. Constructs were then cultured for 4 days to observe self-assembly.

Results
In collagen hydrogels mixed with salts, mineral nodules were observed on the collagen fibrils. When pAsp was added to the collagen solution, mineral nodules were observed to a much lesser extent while FTIR spectra confirmed the presence of phosphate through a similar phosphate stretching vibration peak at $\sim$1035 cm^{-1} in both conditions, indicating intrafibrillar mineralization in pAsp containing hydrogels. The generation of biphasic cellular constructs was achieved by mixing hyaluronic acid into the hydrogels. During 4 days of culture, cells actively contracted both hydrogels to self-assemble into a continuum tissue around the posts.

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Conclusions
We developed a novel one-step methodology to achieve collagen fibrillogenesis, biomimetic mineralization, and cellular embedding. Moreover, we leveraged this methodology to create a self-assembling biphasic bone–tendon-mimetic construct. These constructs will be used to study cell-matrix interactions within healthy and diseased enthesis and have the potential to ultimately contribute to improved therapeutic strategies for enthesis injuries.

Topic: Submit to SYMPOSIUM
Subtopic: Biomimetic and Bioinspired Materials for Regenerative Medicine
Type: Oral presentation

TERMIS25_490 - Genetically modified Potato virus X nanoparticles presenting osteopontin-derived SVVYGLR peptide: a promising approach for functionalizing scaffolds in bone tissue engineering

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Introduction/Objectives
Bone tissue engineering represents one of the strategies employed to address the ever-increasing need for resolving the problem of large bone defects. Both naturally derived and synthetic hydrogels are used to mimic the microenvironment of cells in vivo and guide them towards the desired cell fate. However, the lack of an appropriate combination of biological cues present in the natural extracellular matrix, requires further improvement of hydrogel-based scaffolds. To functionalize hydrogels, plant virus nanoparticles (VNPs) have been proposed as an effective addition to cell-laden 3D hydrogel-based matrices. In a novel approach here, VNPs are used to present high local concentrations of cues for osteogenesis and angiogenesis.

Methods
Potato virus X (PVX) nanoparticles were engineered to present an osteopontin-derived SVVYGLR (SV) peptide sequence on the surface of 100% (PVX-SV) and 25% (PVX-SV2A) of their coat protein units, and their effect on human bone-derived mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs) was investigated. The degree of differentiation of MSCs towards the osteogenic lineage was investigated with Alizarin Red assay, alkaline phosphatase activity assay, and qPCR was used to quantify the change in gene expression of COL1A1, ALP, RUNX2, DLX5, BGLAP (OCN) and BMP2. In the HUVECs study, wound healing migration and Boyden chamber migration were performed in the growth factor-free media and the rate of wound closure and the number of migrated cells were quantified. In addition, qPCR was performed to examine the expression of the genes ITGB1, VEGFR2 (KDR) and VEGFR1 (FLT1). In both cell types, PVX-SV and PVX-SV2A were used at a concentration of 1 ng/ml, corresponding to 3.0 E-14 M. In addition, all experiments were performed with at least three biological donors.

Results
The Alizarin Red assay results showed on average a doubling of the mineralization level of differentiating MSCs when cultured in the presence of PVX-SV and PVX-SV2A. Moreover, osteogenic marker genes were upregulated in the PVX-SV and PVX-SV2A groups. On the other hand, the rate of wound closure by HUVECs was increased between 20 % and 30 % in the presence of the VNPs. The Boyden chamber assay indicated that HUVECs were attracted to the virus-containing media. In addition, qPCR analysis revealed that this VNP concentration was sufficient to induce up to fourfold upregulation of angiogenesis-related genes.

Conclusions
The results show that PVX-SV and PVX-SV2A nanoparticles can be efficiently used in bone tissue engineering to stimulate osteogenesis and angiogenesis simultaneously.

Topic: Submit to SYMPOSIUM
Subtopic: Biomimetic and Bioinspired Materials for Regenerative Medicine
Type: Oral presentation

TERMIS25_1243 - Biomimetic Intervertebral Disc Substitutes: Enhancing Annulus Fibrosus Orientation through 3D Printing and Cryostructuring

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Introduction/Objectives
Low back pain (LBP), recognized by the World Health Organization (WHO) as the most disabling musculoskeletal condition, affects about 80% of the population during their lifetime. Intervertebral disc (IVD) degeneration, a major contributor to LBP, is expected to rise with aging populations[1]. Current treatments like arthroplasty and spinal fusion have high failure rates, highlighting the need for solutions replicating native IVD structure and function. The native IVD consists of a gelatinous nucleus pulposus (NP), which bears spinal loads, and the annulus fibrosus (AF), composed of concentric lamellae with alternating helical orientations (-30°/+30°) supporting the NP[2]. Replicating the AF architecture remains challenging in tissue engineering (TE). This study aims to develop biomimetic IVD substitutes replicating the native disc, emphasizing the AF's structural orientation and the NP's functional properties.

Methods
This study builds on prior work combining 3D printing and ice-templating (cryostructuring) technologies. Previous work showed that 3D-printed magnesium-phosphate cages influenced the growth of cryostructured porous scaffolds, creating geometry-dependent oriented pores[3]. In this study, fused deposition modeling (FDM) was used to develop 3D-printed polycaprolactone (PCL) structures designed to replicate disc shapes. These PCL structures were combined with cryostructured scaffolds made of thiolated hyaluronic acid (HA-SH) and methacrylated gelatin (GelMA), with various cryostructuring conditions tested. Mechanical performance was assessed using a custom dynamic compression setup simulating NP pressure. Additionally, bone marrow mesenchymal stem cells (bmMSCs) were seeded onto the oriented porous scaffolds to assess their alignment, as well as how the pore orientation influences their chondrogenic differentiation and extracellular matrix (ECM) organization.

Results
The combination of 3D-printed PCL structures and cryostructured HA-SH-GelMA blends was successfully developed and optimized under varying cryostructuring parameters. By tuning parameters within 2–4 K/mm, oriented pores were achieved, with angles distributed across ranges that approximate the AF's alternating structure. Current efforts focus on refining the distribution of these angles to better mimic native behaviour. A custom dynamic compression setup was developed to evaluate the scaffolds' ability to mimic native NP pressures, with testing aimed to assess the AF-like layers' capacity to contain the NP-mimicking core. First biological results show that cryostructured scaffolds enhance bmMSCs alignment along the oriented pores compared to cast scaffolds. Ongoing chondrogenic differentiation studies aim to confirm the biomimetic behaviour of the cryostructured scaffolds, aiding their potential for IVD repair.

Conclusions
Preliminary findings indicate that combining 3D printing and cryostructuring enables creation of advanced IVD substitutes with native-like architecture and functional properties. This innovative

approach holds promise beyond IVD field, offering solutions for musculoskeletal TE requiring oriented structures. It represents a step forward in addressing limitations of current therapies for degenerative disc disease.

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Topic: Submit to SYMPOSIUM
Subtopic: Biomimetic and Bioinspired Materials for Regenerative Medicine
Type: Oral presentation

TERMIS25_1454 - Efficient Perfusion Decellularization of Complete Rabbit Rectus Abdominis Muscles

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Introduction and objectives: The repair of complex abdominal wall defects remains a major surgical challenge. The use of synthetic and biological meshes in these defects remains controversial and reconstruction often involves extensive surgical procedures. Decellularized abdominal muscles, containing the native extracellular matrix (ECM) without cellular material, offer a promising alternative to regenerate abdominal muscles. Existing decellularization protocols for skeletal muscle mainly apply immersion methods, which are not suited for the decellularization of larger tissues. The limited size of decellularized matrices from muscle origin limits its potential for regenerative applications. In this study, we developed a method to decellularize complete rabbit rectus abdominis muscles. Given the larger size of this muscle, a perfusion method was established. The objective was to remove nuclear material while preserving the native structure and composition of the ECM.
Methods: The muscle was procured from residual tissue of euthanized rabbits, without systemic heparinization. The inferior epigastric veins were canulated and the rectus abdominis muscle was procured with part of the oblique muscles. Using an in-house developed system, the complete rectus abdominis muscle was perfused ex vivo using a combined immersion-perfusion protocol. The tissue was perfused with 1% sodium dodecyl sulfate (SDS) at room temperature for 24 hours, followed by 2 days of rinsing with demineralized water to remove residual SDS and cellular debris. To further remove DNA, the tissue was perfused with DNase1 for 15 minutes and immersed in DNase1 at 37°C for 3 hours. A final 24-hour rinsing was performed through perfusion with water. Decellularization efficiency was assessed macro- and microscopically and the removal of DNA and preservation of extracellular matrix were quantified using biochemical assays. The decellularized muscles were mechanically characterized through uni-axial tensile testing.
Results: Macroscopically, the tissue appeared white and translucent after decellularization. Removal of nuclear material and cytoplasm upon decellularization was shown by histological stainings (hematoxylin and eosin, DAPI and feulgen staining). More than 90% of the native DNA content was removed upon decellularization and no large-size DNA fragments were visual on gel electrophoresis. The structure of the ECM (hematoxylin and eosin, Martius Scarlet Blue and Alcian Blue staining) and mechanical characteristics (ultimate strength, extensibility and stiffness) remained intact after decellularization.
Conclusions: This perfusion decellularization method allows decellularization of large-size skeletal muscle tissue. Nuclei and cytoplasm were efficiently removed from complete rabbit rectus abdominis muscles, while preserving the ECM structure and the mechanical characteristics. Nevertheless, a low amount of DNA was still detectable. Future in vivo experiments will indicate if this level of DNA removal is sufficient to avoid immune reactions. Decellularized rectus abdominis muscles will be implanted in our validated rabbit model for large-size abdominal wall defects to evaluate its potential to close the abdominal wall, prevent herniation and regenerate the abdominal muscles in cases with complex defects.

Topic: Submit to SYMPOSIUM
Subtopic: Biomimetic and Bioinspired Materials for Regenerative Medicine
Type: Oral presentation

TERMIS25_1456 - Exploring Supercritical CO2 Processing to Generate Highly Preserved Decellularized Placenta for Wound Healing and Regeneration

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Introduction/Objectives
Placenta-derived extracellular matrix (dECM) is a promising material source due to its collagen, growth factors, and cytokines that support cell attachment and proliferation (1). However, traditional decellularization and sterilization methods often compromise ECM bioactivity and leave harmful residues (2), limiting therapeutic potential. To address this, we developed a protocol combining decellularization, drying, and sterilization using supercritical carbon dioxide ($scCO_2$) to enhance bioactivity retention while ensuring scalability and efficiency. Preserved placenta dECM bioactivity could help tackle critical healing challenges, such as chronic wounds, which burden healthcare systems, affecting 1-2% of developed populations (3), and arise from conditions like diabetes, vascular insufficiency, and aging (3). Current treatments, including passive dressings and bioactive therapies, often fail to restore ECM function, leading to prolonged healing and frequent infections (3). Consequently, there is a pressing need for advanced biomaterials that restore wound environment and accelerate healing.

Methods
In this study, placenta ECM was processed using $scCO_2$ combined with sucrose to minimize exposure time to decellularization agents (sodium dodecyl sulfate and sodium hydroxide) while preserving bioactive ECM components. To minimize batch to batch variability equal amounts of 6 human donor placentas were mixed. Decellularization efficiency was evaluated by DNA quantification. Structural integrity was verified through SEM, and the stability was assessed via zeta potential analysis. Additionally, the preservation of key ECM components—elastin, glycosaminoglycans (GAGs), and lipids—was quantified. FTIR analysis was performed to evaluate alterations in the chemical composition. Sterilization was confirmed through a 14-day turbidity test, followed by plating to detect microbial growth. Biocompatibility was assessed using direct contact assays with Human Dermal Fibroblasts (HDFs).

Results
Our results revealed that $scCO_2$-treated dECM achieved improved stability, minimized agglomeration, and ensured complete sterilization, while preserving vital macromolecules essential for tissue regeneration. The optimized decellularization protocol produced a high yield of sterile, dry material, ranging from 42%-52%, and was validated by DNA quantification. DECM spectra revealed reduced lipid and nucleic acid bands and collagen and GAGs preservation. Furthermore, initial *in vitro* studies with HDFs demonstrated the bioactivity of the preserved ECM components, with a notable increase

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in cellular metabolic activity, indicating strong potential for enhancing wound healing and tissue repair.

Conclusions
This work introduces $scCO_2$ as a novel method for decellularizing and stabilizing placenta ECM, preserving bioactivity while enhancing structural properties for chronic wound treatment. *In vitro* studies with HDFs and keratinocytes aim to confirm the regenerative and anti-inflammatory potential of $scCO_2$-treated ECM. Optimized production techniques could provide a safe, human-derived bioactive complex to advance therapies for non-healing wounds.

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Topic: Submit to SYMPOSIUM
Subtopic: Biophysical Therapies for Tissue Regeneration
Type: Oral presentation

TERMIS25_306 - Chondrogenic differentiation in the spotlight: The impact of photobiomodulation on the chondrogenic potential of adipose derived stromal/stem cells

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Introduction/Objectives
Due to their ability to differentiate into the chondrogenic lineage, adipose-derived stromal/stem cells (ASC) are a promising source of therapeutically relevant cells for cartilage tissue regeneration. However, their chondrogenic potential varies considerably between patients. This study explores how photobiomodulation (PBM) can enhance the reliability of ASC differentiation towards a robust chondrogenic phenotype.

Methods
Human ASC from donors of varying chondrogenic potential were treated with LED light at blue (475 nm), green (516 nm), or red (635 nm) wavelengths. PBM was administered either once during the 2D expansion phase or repeatedly during the 3D differentiation phase. Chondrogenic differentiation was assessed via pellet size, glycosaminoclycan and DNA content, histology and gene expression analysis. Donors were classified based on their baseline chondrogenic potential to identify differential responses to PBM.

Results
The effects of PBM were wavelength-dependent and more pronounced when the treatment was applied during cell expansion. Donors were categorized as good or poor responders, with good responders predominantly exhibiting low intrinsic chondrogenic potential. Remarkably, red light PBM resulted in over a twofold increase in pellet size compared to controls, robust collagen type II immunostaining (absent or minimal in untreated controls), and activation of COL2A1 expression. Conversely, in donors with high intrinsic potential, shorter wavelengths (blue and green) produced adverse effects, reducing pellet size, GAG/DNA content, and collagen type II staining. PBM applied repeatedly during the 3D differentiation phase had little to no effect on chondrogenic differentiation, suggesting the question of overdosing via the repeated light exposure.

Conclusions
Our findings demonstrate that PBM can significantly influence ASC chondrogenesis, with outcomes shaped by both treatment parameters and the cells' intrinsic differentiation capacity. The improvement of chondrogenesis in donors with low intrinsic potential highlights PBM as potent tool for cell-based cartilage regeneration. Its cost-effectiveness and ease of use make for an attractive treatment option to enhance the performance of ASC in cartilage tissue engineering.

Topic: Biomaterials
Subtopic: Functionalized, stimuli responsive biomaterials
Type: Oral presentation

TERMIS25_960 - Magnetoelectric membranes to enhance skin wound healing

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Introduction/Objectives
Tissue engineering and biomaterials have notably advanced wound healing research. Composite magnetoelectric materials are gaining interest due to their properties that combine piezoelectricity and magnetostriction. Piezoelectric materials generate electric fields when mechanically stimulated, while magnetostrictive materials deform when subject to magnetic fields. Thus, the combination of both materials allows generating electric fields wirelessly when applying a magnetic field, which offers a non-invasive approach to induce electric stimulation on cells. Here, a highly flexible magnetoelectric heterostructure composed of piezoelectric ZnO nanosheets covered with magnetostrictive FeGa layer embedded in a low Young's modulus elastomer (polydimethylsiloxane) is presented. We demonstrate the *in vitro* effect of the composite on the viability, proliferation and differentiation of two different cell types involved in skin wound healing.

Methods
ZnO/FeGa magnetoelectric membrane cytocompatibility was evaluated using human keratinocytes (HaCaT cells) and human fibroblasts (normal human dermal fibroblasts; NHDF). Cell viability was analysed after 1 and 7 days in culture using Live/Dead Kit (Invitrogen). Magnetic stimulation was used to generate electric fields and analyse their effect on both cell types. A magnetic field of 400 Oe at 100 Hz for 1 h per day, during 7 days, was used. Cell differentiation on magneto-electrically stimulated and non-stimulated membranes was evaluated through immunostaining of differentiation markers. Keratinocytes stratification was analysed through basal cytokeratin 14 and differentiated cytokeratin 10, whereas fibroblasts differentiation was quantified by type I collagen synthesis. In addition, fibroblasts migration was assessed by scratch-wound assay after 24 h.

Results
Results indicated that the ZnO/FeGa membranes were cytocompatible, showing high cell viability (95%) and null adverse effects under magnetoelectric stimulation. In addition, results for magnetoelectric stimulation revealed an interesting effect on keratinocytes stratification and fibroblasts differentiation. The number of keratinocytes with differentiated phenotype (cytokeratin 10) was significantly increased under magnetoelectric stimulation. For fibroblasts, the production of type I collagen and cell migration was also increased under magnetoelectric stimulation.

Conclusions
ZnO/FeGa magnetoelectric membrane demonstrated to be cytocompatible. In addition, the magnetoelectric stimulation enhanced the keratinocytes differentiation and type I collagen synthesis by fibroblasts. The fibroblasts migration on ZnO/FeGa membranes was also increased due to magnetoelectric stimulation. The results highlight the potential beneficial effect of these flexible magnetoelectric membranes for skin wound healing.

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Topic: Submit to SYMPOSIUM
Subtopic: Biophysical Therapies for Tissue Regeneration
Type: Oral presentation

TERMIS25_1109 - A Research Framework for the Multimodal Investigation of Biophysical Treatments for New Indications

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The application of physical therapies has significantly expanded in recent years, moving beyond regenerative uses such as wound and bone healing to addressing a broader spectrum of diseases. This shift requires a comprehensive understanding of the mechanisms of action, safety considerations, and therapeutic potential for novel applications. Our work demonstrates this integrative approach in the context of potential treatments for the eye using various physical therapies. The eye, being a highly sensitive and anatomically complex organ, represents a particularly challenging but promising target for these emerging applications.

Objectives
This study aims to evaluate the feasibility of applying physical therapies to sensitive areas, such as the eye, by developing a framework that combines experimental, computational, and safety-focused methods. The goal is to identify safe and effective treatment parameters while addressing potential risks associated with novel indications.

Methods
The study employs a multi-step approach. In-vitro experiments were conducted to analyze cellular responses to various physical field exposures, such as magnetic, acoustic, or light fields. Direct physical measurements quantified the applied fields, which were then used to simulate their spatial and temporal distribution in the eye's unique anatomy. Established safety parameters were applied to evaluate the potential risks and benefits, focusing on balancing therapeutic efficacy with minimizing adverse effects in the treatment zone.

Results
In-vitro experiments demonstrated promising cellular responses, including enhanced viability and regenerative activity under specific field parameters. Subsequent simulations of the physical fields within the eye revealed localized intensities, highlighting critical areas for dose optimization to prevent adverse effects. Safety assessments suggest that certain parameters may indeed be suitable for the effective treatment of indications of the eye. Further refinement is necessary to address potential risks, particularly in high-sensitivity regions before moving to human subject investigations.

Conclusions
This work highlights the potential of physical therapies for the treating of novel indications, demonstrating the utility of an integrative framework combining experimental, computational, and safety analyses. By understanding the interactions of physical fields with the eye's anatomy and physiology, we provide a pathway for safely and effectively extending these therapies to this sensitive and challenging target. This approach can further guide future developments in expanding physical therapies to other complex and sensitive indications.

Topic: Submit to SYMPOSIUM
Subtopic: Bioprinting in translation to clinic
Type: Oral presentation

TERMIS25_199 - Printable and Implantable hydrogels for translational bioprinting applications

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Introduction/Objectives
To construct an adult human body, a typical composition includes approximately 60-65% water, 16% protein (comprising collagen and other extracellular matrix components), 16% fat, 1% carbohydrate, and a multitude of cells. This composition serves as the baseline when attempting to replicate living soft tissues. In this endeavor, hydrogels have emerged as the most suitable materials, particularly when they are crafted from biomolecules like extracellular matrix supramolecular assemblies. In addition to their composition, soft tissues are distinguished by their mechanical properties, primarily characterized by their Young's modulus, which varies from 1 kPa for brain tissue to 1 MPa for cartilage. Achieving the appropriate mechanical properties is essential and must align with the previously mentioned composition.

Methods
The ongoing pursuit of tissue engineers is to construct 3D living structures that closely mimic the composition and mechanical properties of in vivo conditions. Over the last five years, 10 research groups have collectively dedicated themselves to this pursuit, commencing with the development of a 3D printable hydrogel formulation compatible with the bioprinting of sizable living tissues, then the validation of a clinical grade formulation and finally targeting clinical applications.

Results
We will present how this approach enabled to create cellularized hydrogels with a broad range of mechanical properties while maintaining cell proliferation [1]. This extensive study involved over 19 mammalian cell types sourced from both healthy and pathological tissues, including cell aggregates such as human pancreatic pseudo-islets.
Two examples of clinical applications of the bioinspired hydrogel will be use to illustrate the clinical potentiality:
- An intraoperative bioprinting approach for the reconstruction of severe burn skin using bioink robotic deposition [2].
- A breast cancer reconstruction method using non-cellularized 3D printed hydrogels of tuned properties.

Then, we will introduce to the community an advanced biofabrication platform enabling both bioprinting and cultivation of large 3D tissues within a confined and sterile space [3]. This new concept will be presented together with its unique capabilities.

Conclusions
Most impressively, 19 different mammalian cell types were successfully bioprinted and cultured using the bio-inspired bioink. This includes all cell types tested by our team and collaborators across 8 research institutes, demonstrating that so far, no mammalian cells have failed to thrive and develop within or atop the bio-inspired bioink. Furthermore, the majority of these cells exhibited behaviours consistent with their in vivo counterparts. Complex microstructures and organizations were readily identified, shedding light on intricate morphogenesis mechanisms resulting in the formation of microtubules, micro-vessels, and micro-acini.
It is now evident that microextrusion bioprinting, especially when using bio-inspired bioink formulations, represents a viable avenue for generating a wide range of mammalian soft tissues.

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Topic: Submit to SYMPOSIUM
Subtopic: Bioprinting in translation to clinic
Type: Oral presentation

TERMIS25_608 - 3D bioprinted micro-fat tissue with hydrogels have preserved dimensional stability in vivo

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Introduction/Objectives
High tissue resorption and unpredictable shape stability are well-known problems of autologous fat transplantation that pose significant limitations in reconstructive plastic surgery. We have discovered that the combination of tunicate nanocellulose (TUNICELL) and alginate hydrogels added to micro-fat results in high cell viability and vascularization.

Methods
In this *in vivo* study we have compared shape stability and volume retention of 3D bioprinted micro-fat constructs, crosslinked *ex vivo*, with injected pure micro-fat grafts and injected micro-fat grafts supported by hydrogels with added $CaCO_3$ microparticles for *in situ* crosslinking.

Human fat was harvested by liposuction and microfragmented using a Lipogems® device and then mixed with nanocellulose/alginate to form a hydrogel. In nude mice, we subcutaneously transplanted 3D bioprinted fat constructs, 10 x 10 x 3 mm, shaped as half domes, crosslinked with $CaCl_2$, or injected 250 µl of micro-fat, either pure or with hydrogel with $CaCO_3$ microparticles and glucono-δ-lactone for H^+ induced crosslinking.

Results
3D bioprinted micro-fat, cosslinked *ex vivo*, displayed superior shape stability and better volume retention compared to injected pure fat and injected micro-fat with hydrogels with $CaCO_3$, which relied on *in vivo* crosslinking.

Conclusions
We conclude that addition of hydrogels and 3D bioprinting can improve shape stability *in vivo* and enhance the outcomes of micro-fat transplantation in reconstructive surgery. The efficient crosslinking of alginate played an important role in the dimensional stability of constructs.

Topic: Submit to SYMPOSIUM
Subtopic: Bioprinting in translation to clinic
Type: Oral presentation

TERMIS25_610 - Hydrogels for 3D bioprinting of fat tissue – towards clinical implementation

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Introduction/Objectives
3D bioprinting of human fat tissue has emerged as a promising approach to improve the outcomes of autologous fat transplantation in reconstructive surgery. Until now, only experimental applications have been explored.

Methods
This abstract describes the properties of a standard composition of bioink containing 2.0% nanocellulose and 0.6% alginate, and how these properties may be beneficial for clinical implementation of 3D bioprinted fat.

Results
Cell friendly environment
The bioink, which contains 97.4 % water, creates a very cell-friendly environment. Cell survival in the bioink is excellent.
Printability
The nanocellulose fibers are 2 µ long and 10 nm in diameter, and the bioink has sheer-thinning properties that make bioprinting feasible. The pressure and flow in the printing nozzle reorganize the fibers from random to parallel, thereby reducing stress on the cells themselves. After passing through the nozzle, the ink becomes more stable again.
Dispersing cells
The ink disperses cells well, helping to distribute them evenly in the printed construct.
Shape stability
The shape stability is very good and has been tested *in vivo* for up to one year.
Biocompatibility
The bioink has been tested according to ISO 9000 standards and is as inert as Gore-tex®.
Allows diffusion
According to rigorous investigations, involving MRI, we have found that the diffusion coefficient of the ink is very high, close to that of free water. This allows for excellent transportation of oxygen and nutrients.
Non-degradable
For humans, nanocellulose is non-degradable. This could provide a long-term advantage for preserving a specific 3D shape.

Conclusions
We conclude that a bioink containing 2.0% nanocellulose and 0.6% alginate has several favorable chemical, physical, and biological characteristics that are advantageous in a clinical context.

Topic: Submit to SYMPOSIUM
Subtopic: Bioprinting in translation to clinic
Type: Oral presentation

TERMIS25_1084 - 3D-bioprinting for otorhinolaryngology: Current status and challenges for clinical translation

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Introduction/Objectives
Engineered tissues such as cartilage, bone or fat have the potential to solve significant clinical problems in the head and neck area related to tissue damage due to trauma or tumor resections by providing readily available off-the-shelf solutions. 3D-bioprinting based on novel bioinks might be advantageous to produce replacement tissues in various and personalized shapes for soft tissue reconstruction in the head and neck area. Availability and application in the operating theatre could avoide time consuming and complex cell culture approaches in the lab. Although the scientific basis has been studied in detail, clinical translation is still in its infancy with major hurdles to be taken.

Methods
A literature review was performed through OVID/Web of Science/MEDLINE. Studies reporting on 3D-bioprinting in otorhinolaryngology as well as nasal, auricular and tracheal reconstruction, as well as in situ regeneration and bioprinting were included. Available data include clinical specifics, bioink composition, cell sources, and histological, biochemical and animal model outcomes.

Results
25 studies met inclusion criteria. Nasal, auricular and tracheal cartilage were the main research targets, with mature as well as precursor cells being used. Several different bioinks, such as natural or synthetic polymer based hydrogels have been used for the different applications. Bioprinting technologies for these purposes ranged from microextrusion, droplet and light-based 3D bioprinting to ferromagnetical printing. Several other studies were dedicated to bony reconstruction, as e.g mandibular or skull reconstruction. Only few studies involved animal models, with immunocompetent animal models or clinical studies still missing.

Conclusions
Although 3D-bioprinting as a potential solution to treat tissue defects has advanced significantly in various clinical areas, the technology seems to be underrepresented in studies with otorhinolaryngological background. There is a number of potential applications

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in the head and neck region, such as cartilage reconstruction of nasal and auricular defects or soft tissue reconstruction in the neck where significant benefits for patients and surgeons can be expected. Still, in addition to site specific challenges relative general scarcity of diseases in the head and neck as compared to other medical areas such as orthopedic surgery is a major hurdle for clinical translation in otorhinolaryngology.

Topic: Biosensors & Bioelectronics
Subtopic: Bioelectric stimulation of cells, tissues
Type: Oral presentation

TERMIS25_531 - 3D-Printing of Electroconductive MXene-based Microarchitectures in a Biomimetic Tissue Engineering Scaffold Directs and Enhances Electrical Stimulation for Neural Repair Applications

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Introduction/Objectives
Neurotrauma leads to the disruption of neuronal connections, inflammation and repair-inhibitive scarring and no effective treatment currently exists. The multifaceted therapeutic benefits of electrical stimulation of neural tissues present opportunities to develop multifunctional electroactive tissue engineering scaffolds for neurotrauma repair. We hypothesized that the microstructure an electroconductive scaffold could be designed to enhance delivery of electroactive signalling to drive neural repair. Our aim was to 3D-print tunable conductive microstructures capable of enhanced delivery of electrical stimulation, by functionalizing melt-electrowritten polycaprolactone (PCL) microfibre structures with highly conductive 2D MXene ($Ti_3C_2T_X$) nanosheets. These electroconductive microstructures were then incorporated into a previously developed macroporous scaffold consisting of hyaluronic acid (HA) functionalized with neurotrophic proteins. The capacity of these novel composite scaffolds to enhance the delivery of electrical stimulation to neurons and neurospheres was then examined.

Methods
Multilayer $Ti_3C_2T_x$ MXenes were etched from Al-rich Ti_3AlC_2 MAX phase and dispersed into delaminated sheets and centrifuged to form a concentrated MXene ink. To 3D-print conductive microstructures, a microfibrous PCL mesh was melt-electrowritten (MEW) with a rectilinear pattern containing fibre spacings of 500, 750 and 1000 μm (corresponding to High, Medium and Low fibre densities, respectively) and then functionalized with the MXene ink to form a conductive composite MXene/PCL structure. These 3D-printed microstructures of varying fibre density were then embedded within the neurotrophic HA-based matrix (ECM), containing collagen type-IV and fibronectin, to produce soft, biomimetic (0.6 - 3.25 kPA) composite MXene-ECM scaffolds. The MXene-ECM scaffolds were then seeded with human neuronal cells or murine stem cell-derived neurospheres and electrically stimulated in for up to 7 days.

Results
The electroconductive properties of the MEW MXene/PCL microstructures were highly tunable through changing fiber density and MXene content (0.081±0.053 - 18.87±2.94 S/m) and exhibited electrochemical properties equivalent to metallic electrodes. Neurons exhibited significantly increased neurite length (a 1.47 ± 0.14-fold increase) following electrical stimulation (200 mV/mm) within the MXene-ECM scaffolds. Stem cell-derived neurospheres, electrically stimulated on MXene-ECM composite scaffolds with higher microfibre densities, exhibited a >2-fold ($p<0.05$) increase in maximum axonal length compared to lower density scaffolds and increased expression of βIII-tubulin, a marker of neuronal differentiation.

Conclusions
This study describes the incorporation of a highly conductive nanocomposite microstructure within a neurotrophic and immunomodulatory biomimetic scaffold, producing a novel multifunctional tissue engineering implant capable of driving key repair-relevant behaviours in neurons and neuronal stem cells by enhancing the therapeutic characteristics of exogenous electrical stimulation. Furthermore, these findings indicate that the spatial organization of conductive materials plays an essential role in regulating repair-critical cellular responses to electrical stimulation and demonstrates the broad therapeutic potential of 3D-printable electroconductive scaffolds.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_766 - Influence of construct design and printing parameters on the oxygenation profile of 3D bioprinted soft tissue models using UnaG-based hypoxia biosensor monitoring

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Introduction/Objectives
On the way to manufacture personalized 3D bioprinted tissues and organs for transplantation, the dynamic field of tissue engineering faces numerous challenges. To enable long-term survival and hence successful grafting into the body, an ideal biofabricated tissue should emulate the extracellular matrix and match the mechanical properties of the native tissue as well as ensure that encapsulated cells receive adequate nutrients and oxygen supply within the created 3D environment. Hypoxia, a cellular response to low oxygen conditions, triggers the expression of over 300 genes, making it a fundamental biological mechanism with impact on cell metabolism and vitality. The objective of this study was to assess the oxygenation profile in both the periphery and core of bioprinted soft tissue constructs over time while varying cell concentration and exploring the integration of printed channels.

Methods
For the first time, hypoxia-sensing human adipose-derived stem cells (hADSCs), expressing green UnaG protein under low oxygen levels, were used for soft tissue bioprinting. Spatial distribution and intensity of hypoxic zones were correlated with cell vitality markers. The soft tissue constructs were created through multi-material projection-based 3D bioprinting followed by *in vitro* cultivation over 7 days. To achieve set objectives, cellular metabolic activity in the constructs was evaluated via CellTiter-Glo® viability assay. Moreover, quantification of proliferating and apoptotic cells was achieved by cryotomy and immunohistochemistry. Additionally, a multiphoton confocal microscopy-based 3D imaging protocol was established to investigate the onset and distribution of hypoxia as well as its correlation with the number of dead cells *in situ*, as identified by LIVE/DEAD™ staining kit.

Results
Studying different cell concentrations evidenced the formation of a hypoxic core not only in 10 million cells per mL (10 M/mL) models by day 4 post-bioprinting, but also showed strong centric hypoxia signal in 5 M/mL constructs on day 7 post-bioprinting. As a result, integration of a perfusable channel system into the constructs by multi-material bioprinting focused on 10 M/mL constructs with the strongest hypoxia response. Results depicted generally less pronounced hypoxia reporter response in models with two channels compared to a single one. Notably, channel integration showed more than double metabolic activity on day 1 post-bioprinting among

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10 M/mL conditions, but no difference for the later days as well as no alteration in the proliferating capacity of hADSCs compared to the solid model. Yet, the apoptotic rate declined by almost half on day 7 compared to solid constructs. Additionally, multiphoton microscopy provided a novel perspective on hypoxia and dead cell spatial distribution up to 500 μm into the constructs' core. Results revealed insights on the less hypoxic and apoptotic core in the channelled 10 M/mL constructs while solid constructs showed notably higher level of hypoxia response and double amount of dead cells.

Conclusions
This study illustrated concentration-dependent hypoxia onset in 3D bioprinted soft tissue models as well as positive correlation between hypoxia signal and apoptotic rate in channelled high-cell concentration constructs over 7 days post-bioprinting. Moreover, the findings suggest that hADSC proliferation and metabolic activity may be partially independent of hypoxia reduction.

Topic: Biosensors & Bioelectronics
Subtopic: Bioelectric stimulation of cells, tissues
Type: Oral presentation

TERMIS25_1064 - Opto-nanobiointerface enables multiscale biomodulation

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Introduction/Objectives
Alongside the widely studied pathways of biochemical regulation by chemokines, cytokines and growth factors, one often overlooked but significant influence over the behavior of biological systems is bioelectricity. Voltage gradients among all somatic cells (not just excitable nerve and muscle) control cell behavior, and the ionic coupling of cells into networks via electrochemical synapses allows them to implement tissue-level patterning decisions. Neuromodulation is therefore a potential target for many new therapies for a range of diseases and biological functions. The active modulation of 3D tissue structures through cellular signaling transductions, encompassing thermo-, mechano-, and electrotransduction, has proven highly effective in inducing spatiotemporally controlled, functional tissue maturation.[1] Our current research focuses on advancing biofabrication with remote field stimulation to explore multi-dimensional nano-biointerfaces that synergise the structural induction and the bioelectrical/biochemical signaling to affect cellular behaviours, for biomedical applications in neuromodulation and tissue engineering[2-5].

Methods
Synthesis of hollow sphere graphitic carbon nitride nanoparticles (hg-C3N4 NPs) was done by chemical vapor deposition using silica template. The NPs were characterized by TEM, AFM and Photocurrent measurements. Internalization of NPs were monitored with lysosome co-localization, and intracellular light stimulation with subcellular resolution was demonstrated by calcium flux imaging. At tissue/organ level, the hg-C_3N_4 NPs neuromodulation of retinal tissue was investigated via ex vivo and in vivo studies using intravitreal injection in blind rd10 mice of autosomal recessive retinitis pigmentosa.

Results
The homogeneous hg-C_3N_4 NPs showed photo-responsiveness via photoelectrochemical and photothermal mechanisms. The hg-C_3N_4 NPs can be spontaneously internalized with excellent cytocompatibility. Using a focusing laser, the hg-C_3N_4 NPs enable intracellular optical stimulation with subcellular resolution, inducing calcium transient release in multiple cells and propagation in primary cardiomyocytes and cardiac fibroblasts. At multicellular scale, optical pacing and synchronization of cardiomyocyte beating is achieved facilely by LED. Furthermore, hg-C_3N_4 NPs can be safely injected into mouse eyes, restoring light sensitivity in blind mice. Finally, application of hg-C_3N_4 NPs to porcine retinal tissue *ex vivo* confirmed their modulation capability to reactivate RGCs activity under LED photostimulation.

Conclusions
Taken together, these nanostructured biomimetic semiconductor NPs offer high resolution, leadless optical probing, non-invasive delivery and great biocompatibility, serving as a versatile tool for addressing a range of complex biomedical challenges through subcellular, intercellular and tissue-level photo-modulation across a broad spectrum of scales.

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Topic: Biosensors & Bioelectronics
Subtopic: Bioelectronic devices to monitor cell, tissue growth, development
Type: Oral presentation

TERMIS25_1289 - Functionally linked organoids cultured on suspended MEA webs to model spinal cord injury in vitro

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Introduction/Objectives
Neural organoids are a great advance for in vitro modeling of the central nervous system due to their variety of human-derived cell types, organized structure, and complex electrophysiological activity. Combining two or more organoids into a single system can facilitate the study of more complex biological and electrophysiological interactions. The goal of this project is to promote a functional connection between two organoids cultured on suspended microelectrode array (MEA) webs. This design enables organoids to be cultured at the air liquid interface and ensures limited contact with artificial substrates thereby improving their viability. We aim to create a localized point of connectivity between two organoids by promoting outgrowth across a narrow bridge connecting two MEA webs. The activity of the organoids is recorded with the MEAs to detect spikes and monitor eventual synchronicity between the two 3D networks. Ultimately, this functional connection will be explored as a model for spinal cord injury.

Methods
Thin-film fabrication techniques are used to fabricate MEAs with 150-nanometer-thick platinum electrode tracks sandwiched between two 6-micron-thick polyimide layers. The MEAs are suspended above a culture medium well by mounting them within a stack of acrylic layers. Devices are coated with laminin and Geltrex prior to seeding human induced pluripotent stem cell-derived cortical organoids, which are 2-4 months old and 1-3 mm in diameter upon seeding.

MEAs are connected via custom PCBs to an Intan system for electrophysiological recordings. Custom python scripts are used to analyze spiking. Cell outgrowth from organoids on the MEAs is evaluated via bright field microscopy. To better characterize organoid outgrowth, glass-bottomed microchannels were fabricated via soft lithography of polydimethylsiloxane (PDMS), where one organoid can be seeded on either side of a narrow channel. Electrophysiological activity in these channels was evaluated via calcium imaging.

Results
With the biomaterial coatings, organoids effectively adhere to the MEAs. Axons project and cells migrate from each organoid to form a physical connection between two organoids on a single device. After approximately 7 days, the organoids envelop the MEAs, allowing for recording of electrophysiological activity from within and between the organoids. Activity could be recorded for up to 80 days, and custom software allowed for spikes to be characterized by shape.

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In the glass-bottomed PDMS microchannels, cell outgrowth was also observed to create a physical connection between the organoids. Calcium imaging showed network activity within and between the organoids, indicating functionality of the connection.

Conclusions
Neural organoids were successfully cultured long-term on the suspended MEAs. Cell outgrowth was facilitated by biomaterial coatings and lead to a physical connection of active cells between two organoids. Spikes could be recorded up to 80 days in the device, and the spikes could then be classified into distinct groups. In combination with calcium imaging, spike classification can next be used to characterize the activity of each organoid and assess the synchronicity in their activity. The connection between the organoids will also be characterized by immunostaining and further strengthened via different biomaterial coating strategies.

Topic: Tissue-Specific Focus
Subtopic: Bone
Type: Oral presentation

TERMIS25_509 - Multi-omics profiling of impaired bone healing in diabetes highlights mast cells as targets for biomaterial-enhanced bone regeneration

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Objectives
Type 2 diabetes mellitus (T2DM) significantly hinders the healing of critical-sized bone defects, necessitating innovative biomaterial-based strategies to enhance bone regeneration. This study aims to uncover molecular disruptions in diabetic bone healing through multi-omics profiling to identify potential therapeutic targets for improved personalized treatments.

Methods
Using a diabetic rat model with a critical-sized femoral defect, we conducted a multi-omics analysis over 21 and 42 days post-implantation. Explants from polycaprolactone-supported regenerating bone and contralateral tissue were subjected to a sequential extraction protocol, enabling comprehensive metabolomic and proteomic profiling via mass spectrometry-based approaches. Relative quantitative proteomics utilized tandem mass tag labeling for the analysis on a quadrupole-Orbitrap system. In addition to analysis of central carbon metabolites using GC-MS, quantitative targeted metabolomics was performed using a mixture of flow injection analysis and LC-MS applying the Biocrates Quant MxP500 kit. Differential expression profiles of proteins and metabolites were determined by pairwise comparisons, providing a holistic view of molecular changes occurring during the healing process. Histological and μCT imaging data were integrated with molecular findings to establish correlations between healing progression and clinical outcomes.

Results
This multi-omics workflow identified over 4,000 proteins and 500 metabolites, providing an in-depth perspective into diabetic bone healing. Key observations included impaired structural protein expression critical for soft callus development, disrupted metabolic pathways such as glycolysis and the TCA cycle, and an extended inflammatory phase. Notably, important links were observed pointing to an imbalanced population of mast cells (MCs) within the regenerating tissue of diabetic individuals, with molecular signatures indicating elevated histamine production, clustering of MC proteases, and increased cell density within the regenerating tissue. These findings suggest a dysregulated MC activity in compromised diabetic healing, underscoring their potential as a therapeutic target in biomaterial-assisted bone repair.

Conclusions
This study establishes a novel multi-omics framework for analyzing diabetic bone regeneration, revealing critical molecular disruptions and their clinical manifestations. By identifying mast cells as key contributors to impaired healing, we highlight a promising avenue for targeted intervention in diabetes-compromised bone repair. In an ongoing preclinical study, we are evaluating interventions targeting the observed dysregulated mast cell activity to enhance bone regeneration in diabetes.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_781 - Magnetic hydrogels for bone tissue engineering

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Introduction/Objectives
Bone injuries are a significant healthcare burden with over 4,000,000 bone grafts performed annually to treat skeletal abnormalities, malformations and damage from impact and disease. The 'gold standard' treatment comprises autografted transplants; tissue taken from within the body. However, autograft transplants are limited to preserve function at the donor site, and additional surgery is associated with donor site morbidity and pain. Bone tissue engineering utilises stem cells, 3D scaffolds (natural and synthetic), and growth factors to support bone regeneration as an alternative to grafting. External stimulation, such as magnetic fields, have been applied to further stimulate bone healing. Magnetic field effects on cellular and molecular mechanisms are suggested to be via mechanotransduction. This study introduces a magnetic hydrogel model, utilising an internal magnetic component alongside mesenchymal stromal cells (MSCs) to investigate the mechanism behind magnetic field stimulation for potential therapeutic applications.

Methods
Our magnetic hydrogel model comprises a gelatin matrix functionalised with a photocrosslinkable methacryloyl group and incorporates iron oxide magnetic nanoparticles. MSCs are encapsulated within the magnetic hydrogel as both single cells and spheroids. The model was extensively characterised utilising rheology, scanning electron microscopy, water contact angle and contraction assays. Cell viability within the hydrogel was assessed with a live/dead assay over 28 days. To assess the potential osteogenic response of the MSCs to an external magnetic field, MSCs were encapsulated within the magnetic hydrogel model and exposed to a moderate static magnetic field (370mT) or a dynamic magnetic field for 28 days. Cells were assessed at days 14 and 28, where qPCR was utilised to examine the effect of magnetic fields on osteogenic markers. Stem cell markers were also examined to determine if self-renewal of the stem cell population was occurring.

Results
Magnetic hydrogel characterisation: Mechanical properties, pore size and water contact angle of the magnetic hydrogels were optimised to create an ideal microenvironment for cell encapsulation. Mechanical properties can be adjusted by alerting concentration of gelatin and/or encapsulated MNPs. MSC viability was maintained over 28 days with no significant change.
Magnetic field promotes osteogenesis: Analysis of early osteogenic markers at day 14 revealed significant upregulation of alkaline phosphatase (ALP) and RUNX2 in single cells treated with an external magnetic field, indicating accelerated MSC differentiation toward

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osteogenic lineages. This was mirrored with MSC spheroids, with a significant upregulation of ALP at day 28. Late osteogenic markers also demonstrated increase Osteopontin at day 14 for single cells with a magnetic field, suggesting continued osteogenic differentiation. In the spheroid model, osteocalcin was significantly upregulated. Stemness markers were also elevated at day 14 for single cells, hinting at potential self-renewal within the hydrogel.

Conclusions
The magnetic hydrogel demonstrates the ability to promote osteogenesis in bone tissue engineering models. Given the significant number of bone grafts performed annually, this approach offers a promising alternative to traditional methods. Further research is ongoing to understand the cellular and molecular osteogenic response to a magnetic field.

Topic: Tissue-Specific Focus
Subtopic: Bone
Type: Oral presentation

TERMIS25_1116 - Scaffold guided for bone regeneration and osseointegration of 3D printed bioactive Ca3Si implants

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Introduction/Objectives
Substantial advancements have been made in scaffold-guided bone tissue engineering (SGBTE) in response to the ongoing challenge of reconstructing large segmental bone defects. One of these advancements is the incorporation of silicate bioactive materials into the design of scaffold composites to serve as viable alternatives to traditional autologous bone grafting (ABG). The aim of this study is to evaluate the regenerative capability of 3D-printed scaffolds composed of tricalcium silicate (Ca3Si) combined with a medical-grade polycaprolactone tricalcium phosphate mesh scaffold coated with calcium phosphate (mPCL-TCP-CaP) to reconstruct large segmental bone defects. The objective of this study is to compare the performance of Ca3Si scaffolds combined with mPCL-TCP-CaP mesh in comparison to ABG and mPCL-TCP-CaP scaffolds alone and to scaffolds combined with bone morphogenic protein 2 (BMP-2).

Methods
A 3 cm tibial defect was created in ten sheep and treated using four groups: Group I: Ca3Si + mPCL-TCP-CaP mesh; Group II: Ca3Si + mPCL-TCP-CaP mesh + 1 mg BMP-2; Group III: ABG (control) and Group IV: mPCL-TCP-CaP scaffold (control). Bone regeneration and mechanical properties were assessed after 12 months using X-rays, biomechanical testing, Micro-CT, and histological analyses.

Results
X-ray analysis demonstrated complete defect bridging in experimental groups I and II as early as 3 months post-implantation. The BMP-2-enhanced scaffold (Group II) exhibited superior bone volume and mechanical properties compared to ABG and mPCL-TCP-CaP control groups. Key histological and immunohistochemical findings include well-aligned collagen matrix deposition supporting cortical and lamellar bone formation. Remodelling of secondary osteons and mature lamellar bone was achieved after 12 months of implantation, including remodelling of the intramedullary cavity.

Conclusions
These findings validate the potential of Ca3Si + mPCL-TCP-CaP mesh scaffolds as a scalable, biocompatible alternative to ABG, addressing challenges in availability and post-operative complications. This study highlights the promise of integrating 3D-printing technologies and silicate bioactive scaffolds in SGBTE, paving the way for clinical translation and improved patient outcomes.

Topic: Tissue-Specific Focus
Subtopic: Bone
Type: Oral presentation

TERMIS25_1145 - The co-incorporation of Zn/Cu or Zn/Co ions for improved bone regeneration potential of PEOT/PBT – βTCP composite 3D printed scaffolds

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Introduction/Objectives
Treatment of critical-sized bone defects remains a significant clinical challenge. As an alternative solution to suboptimal gold standard treatment, here, we proposed a combination of a biodegradable polymer possessing bone-bonding properties with bioactive beta tricalcium phosphate (βTCP) and inorganic ions. We choose Zinc (Zn), Copper (Cu), and Cobalt (Co) for their known osteogenic and angiogenic properties. We hypothesised that the combination of Zn/Cu or Zn/Co in the form of a coating of the βTCP particles would stimulate both osteogenic and angiogenic properties of polymer-βTCP composite scaffolds. In addition, we investigated whether the resulting biomaterials would influence the paracrine function of human mesenchymal stromal cells (hMSCs).

Methods
βTCP was coated with either Zn/Cu or Zn/Co through immersion of the βTCP particles in a coating solution containing a 15 mM (low) or 45mM (high) concentrations of the metallic ions. Composites

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were obtained through a combination of the βTCP with poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT) co-polymer in a 50:50 ratio. Composites were additively manufactured into three-dimensional (3D), porous scaffolds by 3D fibre deposition and their osteogenic and angiogenic properties were evaluated using a direct culture with hMSCs as well as in an indirect co-culture with human umbilical vein endothelial cells (HUVECs). Additionally, the influence of the added ions on the paracrine function of hMSCs was assessed by means of the multiplex technology.

Results
The combination of both Zn/Cu and Zn/Co was successfully co-incorporated into the ceramic without changing its chemistry. The culture of hMSCs on composites with inorganic ions enhanced the expression of relevant gene markers. Scaffolds containing low concentrations of Zn/Co increased the expression of *RUNX2*, *OCN*, and *OPN*, while scaffolds with low concentrations of Zn/Cu were most potent at increasing the expression of *ALP*. Furthermore, on the protein level, composites with the addition of a high concentration of Zn/Co resulted in the highest production of ALP and collagen. Regarding scaffolds' angiogenic properties, the presence of ions resulted in increased *VEGFA* expression by hMSCs as well as branching of tubules formed by HUVECs in the case of low concentrations of Zn/Co. Finally, scaffolds containing higher concentrations of added ions resulted in higher secretion of several measured cytokines and chemokines.

Conclusions
Overall, we showed that the addition of metallic ions improved the multifunctionality of the PEOT/PBT-βTCP composite scaffolds. In particular, we found that lower coating concentrations of both Zn/Cu and Zn/Co resulted in more successful upregulation of genes regulating both osteogenesis and angiogenesis, while higher concentrations resulted in higher differences at the protein level. The biomaterials presented in this study might provide an alternative promising solution for treating critical-sized bone defects, which remain challenging in the clinical setting. Furthermore, the scaffolds will be tested in an animal model in an orthotopic critical-sized bone defect as well as in an ectopic implantation.

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1270 - Osteochondral tissue regeneration using multi-cellular gelatin-based implants

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Introduction/Objectives
At present there are no good therapies for the treatment of osteochondral defects, in particular in young and active patients. Tissue engineering strategies involving cell-laden hydrogels offer versatility and are suitable for automated fabrication techniques. In this work we performed an *in vitro* and *in vivo* evaluation of gelatin-based implants containing multiple cell types with the aim of optimizing the conditions for *in vivo* osteochondral tissue regeneration.

Methods
Human iPSC-derived chondrocytes (hiPSC-chons), human periosteum-derived cells (hPDCs) and primary human articular chondrocytes (hACs) were encapsulated in gelatin-based hydrogels. After *in vitro* culture of up to 21 days in differentiation media (containing TGF-β1, GDF-5, BMP-2, BMP-6 and FGF-2), cell viability and metabolic activity were assessed using LIVE/DEAD™ and AlamarBlue™ assays. After mechanical homogenization, gene expression analysis for SOX9, ACAN, COL1A1, COL2A1, COL10A1, RUNX2, OSX, IHH, and CNMD was performed on day 1 and day 21 of *in vitro* culture. To target cartilage and bone regeneration, constructs consisting of hiPSC-chons (1 or 21 days of *in vitro* maturation) and hPDCs (21 days *in vitro* maturation) in gelatin methacryloyl (GEL-MA INX210, BIO INX) were implanted on top of each other (2x 0.5 mm thickness) in a critically sized osteochondral defect (1.6 mm diameter and depth) in nude rats and analyzed after 4 and 16 weeks. Paraffin sections of paraformaldehyde fixed constructs or explants were stained with hematoxylin and eosin, safranin O, alcian blue and toluidine blue as well as immunohistochemistry for collagen type 1, type 2 and human osteocalcin. To quantify the newly formed matrix in the defect site, explants were also analyzed with contrast-enhanced microcomputed tomography.

Results
All the aforementioned cell types can be encapsulated in GEL-MA INX210, (BIO INX) with high viability and with their metabolic activity confirmed up to 7 days *in vitro*. The hiPSC-chons and hPDCs encapsulated in the Gel-MA hydrogel were cultured in the same differentiation medium, yet they displayed a distinct gene expression profile with chondrogenic genes (COL2A1, SOX9, ACAN) more upregulated (25-, 2,5- and 5-fold respectively) in hiPSC-chons and hypertrophic and osteogenic genes (COL1A1, COL10A1, RUNX2) more upregulated (6-, 500- and 20-fold respectively) in the hPDC constructs. Upon orthotopic implantation, histology 4 and 16 weeks post-surgery showed that the hiPSC-chons produced a cartilage-like matrix high in glycosaminoglycans and collagen type II, especially when constructs were implanted after only 1 day of *in vitro* culture. Bone regeneration was consistent over all conditions and by 16 weeks post-surgery, the volume of mineralized matrix formed was not significantly different from healthy control joints. Also, human osteocalcin was observed, indicating the contribution of the humanized implant to tissue regeneration.

Conclusions
Based on our studies, we suggest that immature hiPSC-chon constructs in combination with *in vitro* matured hPDC constructs are favorable for osteochondral tissue engineering. We aim to further investigate the contributions of implant versus host and to explore light-based bioprinting technologies to optimize the interconnection of the two implant types and to better match the dimensions of our implants to the rat model.

Topic: Biomechanics & Mechanotransduction
Subtopic: Mechanotransduction
Type: Oral presentation

TERMIS25_1271 - Altered mineral crystallinity in osteoporotic bone influences osteogenic differentiation and mineralization under estrogen deficiency

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Introduction/Objectives
Postmenopausal osteoporosis (PO) is characterized by alterations in bone tissue microstructure, composition and mechanobiology because of changes in estrogen hormone level [1-2]. In particular, the bone mineral nanostructure is also altered, whereby the proportion of the hydrophilic amorphous layer (HAL) on the surface of nano hydroxyapatite (nHA) particles is reduce in osteoporotic bone [3]. This study aims to (1) develop an advanced 3D in vitro cellular bone model that mimics healthy and osteoporotic mineralized bone models, and (2) investigate how changes in HAL proportion in nHA under estrogen (E) supplementation and estrogen withdrawal (EW) condition influence mineralization and bone regeneration under mechanical loading.

Methods
Novel bone-like proxies composed of platelet-shaped carbonated nHA particles coated with different proportions of HAL, were prepared to represent the crystallinity of healthy (HE: 35%) and

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osteoporotic (OS: 20%) bone tissue. These proxies were incorporated with gelatin (8% wt.) at 12.5% w/w. Pre-osteoblasts (MC3T3-E1) were pre-treated with 17β-estradiol for 7 days and then encapsulated within the gelatin-nHA hydrogels (10^6 cells/ml). Over 42 days, these hydrogels were cultured with estrogen (E) or without estrogen (EW) in static or mechanically stimulated (compression and perfusion) conditions within a bioreactor. TEM, XRD, SSNMR, biochemical assays (DNA, ALP, and calcium content), histological staining (DMP1/actin, Von-Kossa), mechanical testing, micro-CT and Nano-CT scanning were conducted to assess osteogenic differentiation and mineralization.

Results

Nano HA characterization*:** TEM, XRD and SSNMR confirmed the formation of nHA platelet shape particles with different crystallinities. ***Cell proliferation and differentiation: By day 42 actin staining and DMP1 staining confirmed osteocyte differentiation for all groups. Cell number was higher in the OS group compared to the HE group under E (with mechanical loading) and EW, for loaded and static groups. ***Osteogenesis and mineralization:*** ALP and calcium were lower for culture with OS proxy compared to HE under E under mechanical stimulation, but not static conditions, whereas calcium was also significantly lower for OS under EW conditions at day 42. Mineral density (by micro-CT) and bone volume fraction (BV/TV, measured by nano-CT) was lower for the OS than HE under E and mechanical loading, whereas BV/TV was higher in OS than HE in EW at day 42. Compression stiffness was higher in OS than HE under EW at day 42.

Conclusions

In this study, we successfully replicated bone-like carbonated hydroxyapatite nanoparticles with varying hydrophilic coatings, representing healthy and postmenopausal osteoporosis. Our results confirm that an osteoporotic condition inhibits osteocyte differentiation and reduces mineral density significantly under mechanical loading compared to healthy condition, due to increased HA crystallinity and altered hormonal levels.

Acknowledgement: This research is conducted with the financial support of the European Research Council RC consolidator grant (MEMETic: 863795)

References: [1] Naqvi et al, Front Bioeng Biotechnol, 8:601, 2020, [2] Simfia et al, Exp Cell Res, 392(1):112005, 2020, [3] Gamsjaeger, Acta Biomaterialia, 124, 2021.

Key words: postmenopausal osteoporosis, 3D cellular model, crystallinity, estrogen deficiency, mineral density, tissue stiffness

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_1331 - Injectable Hydrogels Mineralized with Sr-Hydroxyapatite Nanoparticles for Advanced Bone Regeneration

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Introduction/Objectives

Bone reconstruction remains a critical challenge in orthopaedic and reconstructive surgery, driven by the complexity of bone defects and fractures and the significant risk of complications, including bacterial infections. This study aimed to develop multifunctional injectable nanocomposite hydrogels incorporating strontium-substituted hydroxyapatite (Sr-HAp) nanoparticles within an ε-polylysine-hyaluronic acid (ε-PL-HA) hydrogel matrix. The objective was to evaluate their physicochemical properties, antibacterial activity, and osteogenic potential for bone tissue engineering applications.

Methods

Sr-HAp nanoparticles were synthesized via chemical precipitation and incorporated into ε-PL-HA hydrogels at varying mass ratios (40–60%). The injectable hydrogels were crosslinked using EDC/NHS chemistry and characterized for injectability, viscoelasticity, enzymatic stability, and ion release. Antibacterial efficacy was assessed against *Staphylococcus aureus* and *Escherichia coli*. In vitro biocompatibility, pre-collagen I synthesis, and alkaline phosphatase (ALP) activity were evaluated using osteoblast (MG-63) and preosteoblast (MC3T3-E1) cell lines.

Results

Hydrogels with Sr-HAp ratios of 40–60% exhibited optimal injectability, stability, and a balance of viscoelastic properties suitable for bone defect filling. The presence of Sr-HAp enhanced the hydrogels' antibacterial activity, achieving significant inhibition of *S. aureus* and *E. coli* growth. Enzymatic degradation studies demonstrated prolonged stability, with degradation times ranging from 5 to 19 weeks depending on Sr-HAp content. In vitro assays confirmed the biocompatibility of the hydrogels and revealed enhanced osteogenic activity, as evidenced by increased ALP activity and pre-collagen I synthesis. These properties were further supported by sustained release of Sr^{2+} ions, promoting osteogenesis and reducing bone resorption.

Conclusions

The developed Sr-HAp/ε-PL-HA nanocomposite hydrogels demonstrate excellent potential as injectable bone-targeted biomaterials. They provide a multifunctional platform combining injectability, antibacterial properties, and enhanced osteogenic capability, offering promising solutions for the regeneration of complex bone defects.

Acknowledgements

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Topic: Tissue-Specific Focus
Subtopic: Bone
Type: Oral presentation

TERMIS25_1375 - Next generation bone adhesives: Bioactive bone void fillers for advanced orthopaedic reconstruction

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Introduction/Objectives

Metal implants commonly used to stabilize bone fragments in trauma surgeries present significant challenges, like infection risk, hardware failure and need for secondary surgery. Additionally, complications like stress shielding and inflammation can limit their long-term clinical success. Current advancements focus on alternative reconstructive materials. Calcium phosphate-based biomaterials have emerged as promising substitutes due to their bioactive potential and improved integration with bone tissue. However, the clinical application is limited because of their suboptimal load-bearing properties after setting. The objective of this research study is to overcome these limitations by developing a mechanically competent and bioactive bone adhesive. By incorporating citrate and demineralized bone matrix (DBM) into phosphoserine modified calcium phosphate formulations, we observed an enhanced bioactivity and mechanical strength. While, citrate incorporation enhanced the shear strength and helped to control setting behavior, DBM imparted bioactivity to adhesive formulation.

Methods

An adhesive system comprising solid and liquid phases was developed. The solid phase consisted of O-phospho-L-serine and alpha-TCP in 1:2 ratio while, the liquid phase consisted 4% Na_2HPO_4. To enhance the properties, adhesive system was modified with either 10% citrate or DBM or both. The properties of adhesive were evaluated through setting time, dissolution behavior, compressive strength and shear strength. The phase was identified with X-ray diffraction (XRD) and micro-architectural properties were analyzed using Scanning electron microscope (SEM). ISO:10993 standards were followed for *in-vitro* assessment of formulation on MC3T3-E1

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osteoblast cell line. The statistical significance was analyzed using one-way analysis of variance (ANOVA) and paired t-test.

Results
Bench-top studies depicted an improved workability and optimized setting time tailored according to clinical needs. It depicted the potential of adhesive formulation for effective orthopedic reconstruction. The initial and final setting time, as determined using Gillmore Needle Apparatus (following ASTM C266 and ISO 9917-1:2007 standards) ranged between 8-13 and 16-21 minutes respectively. The dissolution behavior of adhesive formulation exhibited approximately 40-60% reduced mass loss. Mechanical compression strength was evaluated using ASTM F-451 standard after Simulated body fluid (SBF) incubation. The efficacy in physiological conditions was validated by significant improvement in compressive strength (20.55±2.99 MPa) and shear strength (2.75±0.28 MPa). Enhanced compressive strength and structural stability was validated through XRD and SEM analysis. The results confirmed an increased Hydroxyapatite formation, primary component of bone mineral. Thereby, it promotes mineralization and a potential for improved integration of adhesive. The *in-vitro* results revealed an improved viability, attachment and alkaline phosphatase activity of cells.

Conclusions
The study provides compelling evidence that there is synergistic effect of incorporating citrate and DBM phosphoserine-modified calcium phosphate cements. The significant enhancement in microarchitecture, mechanical properties and *in-vitro* behavior of indigenously developed bone adhesive highlights their promising potential in orthopedic reconstructive applications.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_1559 - A dual-phase thermal sensitive dECM based nanocomposite bioink for cell-laden 3D printing in bone tissue engineering

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Introduction/Objectives
Repairing bone defects remains a significant challenge, and 3D bioprinting offers a promising solution. Decellularized extracellular matrix (dECM) is a common biomaterial for tissue engineering, but its low printability and mechanical strength limit its use in 3D bioprinting. The aim of this study was to to tune the stiffness of dECM to enable fabrication of 3D bioprinted osteogenic constructs with enhanced mechanical and biological properties.

Methods
we developed a thermally sensitive dual-phase bioink based on dECM, incorporating gelatin as a sacrificial material, HAP, and bone marrow mesenchymal stem cells (BMSCs). In vitro studies and a mouse femur defect model were used to assess the biological effects. Additionally, transcriptome sequencing, metabolomics and mass spectrography were employed to evaluate the potential mechanisms of the bioinks in bone tissue engineering and the effects of scaffold structure on cell metabolism.

Results
Rheological analysis revealed improved viscosity and printability with gelatin, supporting dECMbioink printing. And the addition of HAP improved the mechanical strength of the hydrogel. The bioink showed strong biocompatibility and osteoinductive properties, with BMSCs maintaining proliferation and alkaline phosphatase expression. Transcriptomic and metabolomic analyses revealed upregulated cAMP signaling in BMSCs cultured in dECM hydrogel, along with enhanced cysteine, methionine, and collagen-associated metabolic pathways in 3D-printed scaffolds. These findings suggest that dECM hydrogels promote osteogenic differentiation and that scaffold architecture influences cellular metabolism.

Conclusions
The dECM-based bioink osteogenic complex enhanced the osteogenic differentiation of BMSCs and facilitated bone regeneration in critical-size bone defects, leading to great potential in bone tissue engineering.

Topic: Biomaterials
Subtopic: Functionalized, stimuli responsive biomaterials
Type: Oral presentation

TERMIS25_155 - Enhancing Bone-to-Implant Osseointegration via Photocurrent-Driven Immunoregulation and Calcium Influx Activation in Macrophages

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Introduction/Objectives: The foreign body reaction is a natural response to an implant, involving complex cellular and molecular events that determine implant integration. Macrophages, among the first immune cells to arrive, initiate acute inflammation, recruit mesenchymal stem cells (MSCs), and start regeneration. This proinflammatory response peaks within 24-48 hours post-implantation. If immune self-regulation is compromised, uncontrolled inflammation can become chronic, leading to implant failure. Implant loosening accounts for over 10% of failures. Thus, promptly restoring the osteoimmune microenvironment after initial inflammation is crucial to prevent chronic inflammation and ensure successful osseointegration.
Methods: Hydroxyapatite, a widely used biocompatible orthopedic biomaterial, was utilized to fabricate an excitable surface via laser cladding on titanium substrates. The high-temperature cladding process facilitated the decomposition of HA and its subsequent interaction with the Ti substrate. A comprehensive surface characterization was conducted using X-ray diffraction, X-ray photoelectron spectroscopy, and electron spin resonance spectroscopy to investigate the properties of the excitable surface. The photocatalytic activity of this surface under 808 nm near-infrared (NIR) irradiation, along with the underlying mechanisms, was examined using electrochemical measurements and hybrid density functional theory calculations. The impact of the photocurrent generated by the excitable surface under NIR light on the modulation of the osteoimmune microenvironment was evaluated through both in vitro and in vivo studies to assess its effectiveness in promoting osseointegration.
Results: A defective engineered n-n heterojunction between $CaTiO_3$ and TiO_2-Vo ($CaTiO_3$-TiO_2-Vo) was formed on the excitable surface. Under NIR light irradiation, this surface generated a photocurrent that accurately directed macrophage polarization, thereby alleviating acute inflammation in the early post-implantation phase. Transcriptomic analysis and in vitro studies indicated that photoelectric signals initiated an increased calcium influx in macrophages under NIR irradiation, which altered the expression of calcium/calmodulin-dependent protein kinase kinase 2 and calcium/calmodulin-dependent protein kinase I to inhibit M1 macrophage polarization. This resulted in a favorable osteoimmune microenvironment that enhanced the recruitment of MSCs and osteogenesis, thereby accelerating osseointegration within 14 days following implantation.
Conclusions: In summary, we designed an excitable surface capable of remotely manipulating the osteoimmune microenvironment to facilitate osseointegration. Upon NIR irradiation, the excitable surface generated an intensified photocurrent, which activated voltage-gated calcium channels to instruct macrophage phenotype switching on the implant surface, favoring M2 macrophage dominance. The cytokine profile included reduced pro-inflammatory cytokines (TNF-α and iNOS) and increased anti-inflammatory cytokines (ARG-1 and IL-10). This favorably modulated osteoimmune microenvironment significantly accelerated osseointegration in a tibia defect in an in vivo animal model as early as 14 days post-implantation.

Topic: Biomaterials
Subtopic: Micro-&nanoscale materials
Type: Oral presentation

TERMIS25_320 - Physical and chemical niche of human growth plate for polarized bone development

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Introduction/Objectives
Deciphering how polarized mineralization patterns in the growth plate (GP) contribute to long bone formation is a significant challenge in understanding bone development throughout the growth phase. Previous studies have extensively revealed the unique contribution of disparate cell types within the GP in guiding long-term bone elongation. However, the polarized mineralization pattern and its underlying mechanism in the extracellular matrix of human GPs have been rarely explored.

Methods
The human GPs were obtained from amputation samples. The GP mineralization processes were defined by Scanning Electron Microscope (SEM), Focused Ion Beam-SEM (FIB-SEM), Raman microscope, Atomic Force Microscope (AFM), X-ray tomography (XRM), Transmission Electron Microscope (TEM), Cryo-TEM and 3D tomography reconstruction. The regulatory proteins were revealed by proteomics of different part of GP samples.

Results
By employing high-resolution analytical techniques, we reveal that the GP-epiphysis interface displays a sharp transition in tissue modulus, acting as a "protective shell" for the underlying GP, whereas the GP-metaphysis interface exhibits a gradual modulus increase, enabling efficient load redistribution to metaphysis. This mechanical microenvironment drives unique microstructural and compositional transformations from GP to epiphysis and metaphysis. Notably, the GP-epiphysis interface acts as a mineralization inhibition zone while the GP-metaphysis serves as a mineralization promotion zone, orchestrated by a complex network of proteins. Proteins such as SPP1 and AHSG at the GP-epiphysis interface inhibit mineralization, forming a defense line; while ENPP1 and ALPL coexisted with SPP1 and AHSG at the GP-metaphysis promote a sequential nucleation. Such polarized mineralization patterns maintain the homeostasis of GPs and promote bone polarized elongation. Replicating this process in vitro, we synthesized stable amorphous calcium phosphate which showed highly controlled transformation to hydroxyapatites.

Conclusions
Taken together, this study gives unique insights into understanding the polar mineralization patterns in developmental human GPs and presents a potential blueprint and strategy for a controlled biomineralization process.

Topic: Tissue-Specific Focus
Subtopic: Liver
Type: Oral presentation

TERMIS25_539 - Altered vitamin D metabolism in MASLD-associated bone dysfunction: A novel 3D in vitro liver-bone model

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Introduction/Objectives
Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), a condition affecting millions globally, is closely linked to liver cirrhosis and disruptions in bone metabolism. However, the underlying mechanisms remain poorly understood. Vitamin D is crucial for bone homeostasis, and the liver regulates its metabolism; this study explores how altered vitamin D pathways in MASLD contribute to impaired bone homeostasis.

Methods
Our 3D micro-organoid model uses HepaRG cells as hepatocytes, LX-2 cells as stellate cells, and Human Umbilical Vein Endothelial Cells to mimic endothelial functions. To induce MASLD features, the micro-organoids were treated with a Palmitic/Oleic acid cocktail. Fatty acid accumulation was quantified and visualized using Bodipy staining. Secreted proteins indicating liver damage were evaluated using Dot blot. Gene expression analysis targeted epithelial-to-mesenchymal transition (EMT). Western blot analyses were performed to measure the protein expression of key cytochrome P450 enzymes (CYP). Enzyme-linked immunosorbent Assay (ELISA) and RT-PCR were performed to assess the metabolic capacity and gene expression of different vitamin D pathway markers.

Our well-established 3D scaffold bone model consists of SCP-1 cells as osteoblast-like cells and THP-1 cells as osteoclast-like cells; were treated with conditioned medium from MASLD liver micro-organoids. Alkaline phosphatase (AP) activity and Tartrate-resistant acid phosphate (TRAP) activity assays were performed to assess bone-forming and bone-resorbing cells' activity. The mineral content and stiffness of the bone scaffolds were analyzed using computer tomography.

Results
MASLD liver micro-organoid model demonstrated significant lipid accumulation, confirmed by Bodipy staining. Gene expression analysis showed dysregulation of fatty acid metabolism markers, validating the disease model. Accumulation of fatty acids was associated with increased secretion of AP, indicating elevated liver damage, and increased Procollagen Typ 1 N-terminals Propeptide, suggesting enhanced ECM production. Gene expression analysis showed higher levels of EMT markers, such as SNAIL and N-cadherin, indicating a shift toward a mesenchymal phenotype. Decreased expression of CYP1A2, CYP3A4, and CYP2C9 paralleled the patterns observed in MASLD patients. Enzymes crucial for vitamin D metabolism, including CYP27A1 and CYP2R1, were downregulated, further supported by RNA sequencing data from MASLD patients obtained from the National Center for Biotechnology Information (NCBI; PRJEB58091). Furthermore, 25-Hydroxy vitamin D ELISA also confirmed vitamin D metabolism impairment.

Our data suggests that exposure of bone scaffolds to vitamin D-reduced conditioned media from MASLD organoids results in the decreased activity of bone-forming cells and increased bone-resorbing cells. Hence, this leads to decreased stiffness and mineral content of the bone scaffold.

Conclusions
This project is the first developed human-derived liver-bone in vitro model that allows the study of the MASLD's impact on bone health and provides a translational platform for developing novel therapies. Integrating liver organoids with bone cells enables the study of liver-bone crosstalk and offers a promising alternative to animal models. This approach could advance drug discovery, enabling personalized and more effective treatment strategies for patients with MASLD.

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_714 - Modeling human endosteal bone marrow niches from induced pluripotent stem cells in Xeno-free conditions

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Introduction/Objectives
The bone marrow (BM) hosts hematopoietic stem and progenitor cells (HSPCs). While the majority of HSPCs are located in perivascular niches far away from the endosteal bone surface, a fraction of HSPCs with important functions in non-steady state hematopoiesis localize in perivascular endosteal niches according to murine studies. However, developing a human model of the endosteal BM niche to study this complex microenvironment remains as a significant challenge. This limitation hinders our understanding of the complex interactions between this niche and HSPCs, which depends on the precise temporal and spatial arrangement of heterogeneous cells - such as mesenchymal stromal cells, osteoblasts, and vascular cells.

This study aimed to generate a 3D *in vitro* model of the human endosteal BM niche by following a chemically-defined and xeno-free protocol to directly differentiate human induced pluripotent stem cells (hiPSCs) into osteoblasts and vascular cells.

Methods
To produce this so-called engineered vascularized osteoblast niche (eVON), we used small molecular induction to guide in paralell the differentiation of hiPSCs toward osteoblasts and vascular cells (endothelial cells and pericytes in a 3D bone-like hydroxyapatite scaffold.

Results
The eVON was characterized by the presence of human mesenchymal stromal cells (MSC) as well as the expression of vascular and osteoblastic markers using different techniques such as immunostainings, flow cytometry, scanning electron microscopy (SEM), and real-time quantitative PCR (RT-PCR). After 31 days of culture, immunostaining for CD34, a marker for human vasculature, confirmed the presence of vascular networks integrated into dense osteocalcin osteocalcin-positive osteogenic matrix in eVON. Flow cytometry data revealed that 1-2% of cells isolated from the eVON express the pan-endothelial cell marker CD31, and the remaining cells express MSC markers such as CD146 (60%) and CD271 (30-40%). Additionally, we assessed the *in vivo* stability of the eVON by implanting it ectopically in immunodeficient mice. Our results showed the survival of eVON after 6 weeks *in vivo* and a good integration of human cells in the host murine tissue. Furthermore, we showed that eVON can sustain human hematopoietic progenitors better than avascular osteoblastic niches (eON), revealing the importance of vascular structures in this context. To further validate our protocol, we generated eVON using three different hiPSC lines: WTC11, WiBJ, and 409B2. Single-cell transcriptomic analysis confirmed the complex composition of the eVON, including different subtypes of MSCs, vascular smooth muscle cells, endothelial cells, epithelial cells and even neural cells. Additionally, we could identify across different cell clusters the expression of specific gene key for the maintenance of HSPCs in their niches, such as *CXCL12*, KITLG/SCF and VEGFA. Interestingly, we found that eVON generated from different cell lines supported distinct hematopoietic lineages. Finally, we provided a proof-of-principle of the applicability of the model for pharmacological perturbations by targeting VEGFA signaling and analyzing the effects on the vasculature.

Conclusions
In conclusion, we have developed a robust protocol to generate standardized eVON from hiPSCs that can be applied to study human hematopoiesis in human endosteal BM niches.

Topic: Biomechanics & Mechanotransduction
Subtopic: Physiological forces and functions
Type: Oral presentation

TERMIS25_1059 - Multiscale Methodologies for Improved Analysis of Fracture Healing

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Introduction/Objectives
A bone fracture represents a prevalent bodily injury. The recovery process is characterized by a physiological complexity in which both biological and mechanical dimensions are intricately intertwined. Computational models based on phenomenology for tissue regeneration and bone healing have achieved only partial success in forecasting experimental findings. Proposed mechanoregulation algorithms aim to explore potential connections between mechanical stimulation and the differentiation of cells and tissues, especially in the context of bone healing. The mechanical stress on the tissue formed in the fracture gap is an important factor in fracture healing. However, this is largely disregarded in the selection of postoperative rehabilitation measures for the follow-up treatment of osteosynthetic fractures. At present, postoperative treatment is based exclusively on radiological snapshots and the subjective assessment of treating physicians.

Methods
A coupled bidirectional approach between macroscopic stresses and microscale mechanoregulation cell differentiation is presented in this paper. For the macroscale, a Multibody simulation (MBS) is used to gain insights into the body, while on the complex material behaviour and fracture healing process is analyzed via Finite Element Methods (FEM). The bidirectional coupling of MBS and FEM exploits the strengths of both methods and offers great potential for analyzing the deformations and stresses in the fracture gap, taking into account the kinematics and kinetics of the body. The results of a MBS are used as input for a FEM, and the resulting multi-scale approach is applied to the example of the treatment of a proximal femur fracture using a proximal femoral nail (PFN). In the first step of the multi-scale approach, static and dynamic loading of the femur in bipedal stance and during fully loaded gait are determined within two MBSs using two musculoskeletal models. Subsequently, these determined loads are implemented as boundary conditions (BCs) for four FE models representing different integrity states of the femur or femur-PFN construct. In the second step of the multi-scale approach, stresses and deformations of tissue located in the fracture gap caused by these loads are determined within four FEAs. Stresses and deformations are then interpreted with threshold values from three mechanoregulatory theories, allowing an evaluation of rehabilitation measures concerning their mechanobiological fracture healing conditions.

Results
Due to model assumptions made, evaluation significance remains limited; however, comparing threshold values with determined stresses allows predictions about fracture healing under two rehabilitation measures. According to one theory, stresses and deformations caused by loads during bipedal stance promote fibrous connective tissue formation only in advanced healing states, posing risks for impaired fracture healing.

Conclusions
With this developed multi-scale approach alongside more realistic modeling of implant-bone contact—individualization of musculoskeletal models—and modeling biological processes involved in fracture healing—rehabilitation measures could be evaluated more comprehensively in future research. This would enable patient-specific rehabilitation programs that consider biological and mechanobiological conditions for effective fracture healing.

Topic: Stem Cells
Subtopic: Regeneration, repair of tissues & organs
Type: Oral presentation

TERMIS25_1166 - Profiling critical bone fractures in axolotls: a cellular and molecular perspective

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Introduction/Objectives
Fractures are common traumatic injuries. While small, aligned fractures typically heal by fibrocartilaginous callus formation, large and

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complex fractures often result in non-union (pseudoarthrosis) in 5–10% of cases. This significantly impacts patients' mobility and quality of life, imposing substantial clinical, economic, and social burdens. To study fracture non-union, the critical size defect (CSD) model is utilized. Axolotls, known for their remarkable limb regeneration via a transient progenitor pool, blastema, paradoxically cannot heal CSD. Our study investigates if soft connective tissue (SCT) cells migrate to the CSD site and explores the factors that could enable SCT cells to participate in bone regeneration.

Methods
To evaluate SCT cell migration and potential cartilage contribution in the CSD, we transplanted fluorescently labeled SCT cells into wild-type axolotls prior to fracture surgery. Bulk and single-cell transcriptomics were used to identify the factors influencing SCT cell dedifferentiation into blastema cells compared to the CSD.

Single-cell atlases of blastema and CSD encompassed SCT cells, epidermis, macrophages, neutrophils, and other cell types. To examine if blastema-specific expression has an impact on cell activation and migration, data was used to model gene regulatory networks (GRNs) of SCT cells and identify ligand-receptor interactions using the NicheNet algorithm.

Results
Fluorescent labeling showed SCT cell migration to the CSD gap, with some cells expressing the cartilage progenitor marker SOX9. Proliferation rates were significantly lower in CSD than in the blastema.

Bulk and single-cell transcriptome analysis highlighted altered gene expression profiles associated with proliferation and dedifferentiation. Differences in interactions between SCT cells, wound epidermis, and macrophages were identified. Notably, early blastema (3–5 days post-amputation) showed strong interactions between SCT cells and epidermal or macrophage populations, absent in the CSD.

NicheNet analysis demonstrated the importance of epidermis-derived ligands for SCT cell activation in blastema, with blastema epidermal population exhibiting a distinct wound epidermis signature. Transcriptome-based GRN inference revealed shifts in transcription factor hubs, including WNT and TGF-β pathways. Differential involvement of TCF7L2 and LEF1 transcription factors, along with WNT pathway components, was implicated in regulating SCT cell behavior in regenerative versus homeostatic contexts.

Conclusions
Our findings demonstrate that SCT cells migrate to axolotl CSD but fail to proliferate or form blastema tissue. This failure is attributed to insufficient interactions between fibroblasts and wound epidermis, along with inadequate TGF-β and WNT signaling in CSD. Rewiring GRNs in the CSD environment to induce a blastema-like phenotype holds potential for promoting bone regeneration and bridging critical size defects.

Topic: Submit to SYMPOSIUM
Subtopic: Extracellular matrix-inspired biomaterials for cartilage and intervertebral disc regeneration
Type: Oral presentation

TERMIS25_317 - Engineering a 3D-printed biomimetic model of the intervertebral disc

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Objective
Lower back pain is a epidemiological and socioeconomic problem with a global prevalence of 80% [1]. Although intervertebral disc (IVD) degeneration is subject to intensive research, pathology remains poorly understood. Recent advances in 3D- and bioprinting have facilitated biofabrication of IVD models that imitate native tissue structure arrangement of a central gelatinous nucleus pulposus (NP) and surrounding lamellar annulus fibrosus (AF) [2,3]. However, existing models fail to replicate the mechanical strength of native tissue, lack the intricate 3D structure of extracellular matrix (ECM) and do not capture the dynamic changes associated with degeneration. This work aimed to develop a controlled and reproducible 3D model that could serve as an *in vitro* platform for studying IVD pathology and evaluating new therapies in future research. To accomplish this, 3 ECM-based biomimetic bioinks were created and characterised to bioprint a scaled IVD model with clearly defined NP, inner (i)AF and outer (o)AF regions.

Methods
Bovine caudal discs obtained from a local butcher were characterised for dimensions, mechanical and biochemical properties. Discs were measured to establish print model dimensions; compressive and tensile testing was performed to acquire target mechanical properties and NP, iAF, and oAF were biochemically investigated to determine bioink compositions. Simultaneously NP and AF ECM was isolated by decellularisation (0.2M NaOH, cryomilling, 2U/ml Benzonase, pepsin digestion, salt precipitation). ECM was methacrylated (MA) together with elastin and chondroitin sulphate (CS; a major glycosaminoglycan component of IVD). Tissue-specific bioinks were prepared according to native proportions of ECM, CS and elastin and characterised prior to bioprinting using a custom HYREL 3D-printer.

Results
NP, iAF, and oAF tissue-specific ECM-based bioinks were successfully fabricated according to native ECM composition, comprising of ECM-MA, CS-MA, and elastin-MA in relevant concentrations. Bioinks could be 3D-printed through 23G and 25G nozzles with high reproducibility and fidelity to the original G-code. Printing induced collagen fibre alignment which mimics native AF lamellar organisation, a prerequisite for tensile strength. Printed constructs were successfully photocrosslinked by added photoinitiator (LAP; 0.25% w/v) on exposure to blue light (405 nm). Crosslinking duration impacted mechanical strength, allowing tuneable mechanical properties in bioinks and final 3D-printed structures. Encapsulated primary NP cells demonstrated excellent viability post printing.

Conclusion
Comprehensive characterisation was performed to evaluate the bioinks' composition, stability, and mechanical properties, ensuring they closely replicate the ECM environment. While further investigation is required to fully elucidate cellular responses and behaviour following bioink encapsulation and bioprinting, these findings establish a solid foundation for engineering a complex, ECM-based, scaled 3D-bioprinted IVD model. This model provides a promising platform for investigating cellular interactions within a tissue mimetic environment, advancing future research into IVD pathology and enabling evaluation of innovative therapeutic strategies.

Acknowledgement
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Topic: Tissue-Specific Focus
Subtopic: Intervertebral disc & spine
Type: Oral presentation

TERMIS25_355 - Promoting Intervertebral Disc Fusion: The prostaglandin E2 receptor 4 agonist KMN159 acts synergistically with BMP2 and L51P to induce osteogenic differentiation of human annulus fibrosus cells

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Introduction
Spinal fusion surgery is often performed to restore proper spinal alignment, but it presents notable challenges, particularly in older patients with comorbidities. This study aims to identify factors that promote the fusion of intervertebral discs, focusing on minimally invasive approaches for spinal surgery. Specifically, we examine a novel method to induce osteogenic differentiation in annulus fibrosus cells (AFCs), using a combination of bone morphogenetic protein-2 (BMP2), L51P (a BMP2 analog), and KMN159 (a prostaglandin E2 receptor 4 agonist, which modulates inflammatory processes).

Methods
Primary human AFCs (n=4) were stimulated with BMP2, L51P, and KMN159 and cultured for 21 days. Cell viability was assessed to detect cytotoxic effects following stimulation. At 7, 14, and 21 days, osteogenesis was evaluated by measuring the transcription levels of bone-related genes: alkaline phosphatase (ALP), Runt-related transcription factor 2 (RUNX2), bone gamma-carboxyglutamate protein (BGLAP, also known as osteocalcin), secreted phosphoprotein 1 (SPP1, also known as osteopontin), osterix (SP7), and type I collagen (COL1). In parallel, BMP antagonists such as Noggin, Gremlin 1, and Chordin were quantified using quantitative polymerase chain reaction (qPCR). On Day 14, ALP activity was measured at the protein level, and on Day 21, histological staining with alizarin red (ALZR) was performed to detect calcium deposits, indicating mineralization.

Results
KMN159 had no discernible impact on the cell viability of AFCs but demonstrated weaker osteogenic effects on AFCs compared to BMP2. Specifically, fold changes in the expression of osteogenic markers on Day 21 showed BMP2 had a stronger effect than KMN159 in promoting the expression of genes such as ALP (2.828 vs 2.516), RUNX2 (4.199 vs 0.863), BGLAP (2.035 vs 0.7028), SPP1 (6.407 vs 1.078), SP7 (42.20 vs 1.094), and COL1 (1.664 vs 0.9518). When KMN159 was combined with BMP2 and L51P, there was a significant increase in ALP expression in AFCs ($P < 0.05$), suggesting enhanced osteogenic differentiation. This result was corroborated by increased ALP protein activity on Day 14 and intensified calcium deposition detected by ALZR staining on Day 21. Although other bone-related markers did not show statistically significant changes, an upward trend in expression was observed after 21 days. KMN159 had minimal impact on BMP antagonists (Noggin, Gremlin 1, and Chordin), indicating its minimal influence on their expression.

Conclusions
Our results suggest that the combination of KMN159, BMP2, and L51P can successfully induce osteogenic differentiation in human AFCs. This combination may serve as a promising minimally invasive approach to enhance spinal fusion without the need to remove the intervertebral disc, offering potential new treatment options for patients suffering from lower back pain.

Topic: Tissue-Specific Focus
Subtopic: Intervertebral disc & spine
Type: Oral presentation

TERMIS25_387 - Targeting homeostasis restoration in nucleus pulposus cells of intervertebral discs via miRNA-paired delivery supported by human platelet lysate

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Introduction/Objectives
Degeneration of the intervertebral disc (IVD) is associated with a dysregulation of anabolic : catabolic homeostasis within the nucleus pulposus (NP) region of the disc [1]. Our group has established that delivering microRNAs (miRNAs) -149-5p mimic and 221-3p inhibitor in tandem with the cell-penetrating peptide GAG-binding enhanced transduction FGF2B-LK15-8R (GET-FLR) has a dampening effect on catabolic enzymes. Although evidence indicates a shift towards enhancing anabolic potential, this effect remains incomplete and may benefit from additional supplementation [3]. Human platelet lysate (HPL) is rich in growth factors and anti-inflammatory cytokines, whereby extracellular matrix (ECM) proteins such as aggrecan (ACAN) and collagen type II (COL2) can be stimulated [4]. This study aims to combine the delivery of miRNA-149-5p mimic and miRNA-221-3p inhibitor with HPL in rat nucleus pulposus monolayer and organ culture models of intervertebral disc degeneration to stimulate repair.

Methods
After determining an appropriate HPL dosage, rat nucleus pulposus cells were transfected with FLR-miRNA-149-5p mimic and miRNA-221-3p inhibitor in monolayer, and supplemented with 10% HPL, or cultured in serum free (SF) media. Ex vivo organ culture entailed isolating the top three caudal discs from a rat tail and inducing mild NP degeneration for 7 days via chondroitinase ABC 0.025U injection. Treatments of 10% HPL, miRNA pair, and 10% HPL + miRNA pair were delivered, alongside non-transfected (NT) and untreated controls, and cultured for 14 further days.

Results
In monolayer, HPL stimulation at 10% significantly upregulated COL2 protein expression compared to the untreated control ($p = 0.036$), with increases of ACAN also demonstrated. HPL + miRNA pair significantly upregulated COL2 ($p = 0.015$), with ACAN approaching a two-fold increase. Proliferation was also strongly increased ($p = 0.004$) in the HPL + miRNA pair group. Ex vivo organ culture demonstrated increased glycosaminoglycan (GAG) staining in the 10% HPL + miRNA pair group across all donors, comparable to that of the healthy non-degenerated control.

Conclusions
The addition of HPL to dual miRNA delivery demonstrated a stimulation of ECM proteins in both monolayer and ex vivo organ culture models. Future studies will aim to evaluate the effects on catabolic markers to better understand their role and potential in restoring homeostatic balance within the nucleus pulposus of the intervertebral disc. Additionally, incorporating degenerated human nucleus pulposus cells will seek to enhance our understanding of the therapeutic potential of this combined approach.

Acknowledgements
This work was supported by the European Research Council (ERC-2019-CoG-864104: INTEGRATE).

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Topic: Submit to SYMPOSIUM
Subtopic: The role of osteoblasts/osteoclasts in bone cell interaction models in vitro - tools for 3Rs-compliant analysis of drugs and biomaterials in the bone context.
Type: Oral presentation

TERMIS25_510 - 3D printed co-cultures of osteocytes and osteoclasts as in vitro bone model

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Introduction/Objectives
Bioprinting is a versatile tool to generate heterogeneous and functional tissue models with a spatially defined cell distribution. In vitro bone models usually lack an osteocyte component as these cells are difficult to isolate and cannot be expanded. The aim of the present study is to generate 3D bioprinted human osteocyte (Ocy)-laden constructs, co-cultivated with human osteoclasts (OC) to analyse their cross talk in a spatial arrangement, which is close to human bone. Moreover, we aimed to establish an animal-free experimental setup, meeting the 3R principles by omitting fetal calf serum (FCS) in our model.

Methods
Primary pre-osteoblasts, isolated from human femoral heads were bioprinted in an alginate/methylcellulose/human plasma-based bio-ink [1]. The constructs were cultivated for 14 days without addition of FCS, in the presence of 2 % platelet lysate (PL) and 100 ng/mL bone morphogenetic protein 2 (BMP-2) for differentiation into osteocytes and characterized by gene expression analysis. OC were differentiated from peripheral blood mononuclear cells (PBMC), again in the presence of PL instead of FSC [2] and seeded either directly onto the bioprinted constructs or in close vicinity. Constructs were further cultivated in a mixed medium allowing maintenance of both OC and Ocy phenotype. Morphology of the cells was examined by fluorescence microscopy. Cell culture supernatants were used to quantify the activity of osteoclast-specific enzymes tartrate-resistant acid phosphatase (TRAP) and cathepsin K as well as the secretion of osteocyte-specific proteins osteocalcin, osteoprotegerin (OPG), receptor activator of NF-κB ligand (RANKL) and sclerostin by ELISA.

Results
Osteocytes were successfully differentiated in bioprinted constructs, showing dendritic morphology and expression of osteocyte markers podoplanin, osteocalcin, sclerostin and dentin matrix protein 1. The addition of BMP-2 significantly increased the expression of osteocyte-specific genes, especially of the late osteocyte markers. Osteoclasts were predifferentiated in the presence of 10 % PL. After successful detachment of multinucleated OC from the ultra low binding surfaces, the cells were seeded onto the bioprinted strands. Activity of OC-specific enzymes TRAP and cathepsin K was detected in the cell culture supernatants. No additional supplementation of the co-cultures with RANKL was necessary to maintain OC activity.

Conclusions
The proposed in vitro model can be performed without using FCS, both for isolating and expanding the cells and during cultivation of the constructs, which is not only an important step to reduce animal use, but also to overcome non-physiological interactions of the examined bone cells with bovine proteins and growth factors. It is planned to additionally involve human osteoblasts into the 3D printed in vitro bone model to generate an even more bone-like model, which can help to reduce animal experiments for testing of drugs and bioactive compounds in bone.

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Topic: Tissue-Specific Focus
Subtopic: Intervertebral disc & spine
Type: Oral presentation

TERMIS25_712 - A degradable nanofibrous scaffold of poly (ε-caprolactone-co-lactide) for annulus fibrosus regeneration

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Introduction/Objectives
The intervertebral disc consists of a gelatinous core (nucleus pulposus, NP) surrounded by a fibrous ring (annulus fibrosus, AF). NP herniation is a significant cause of low back pain, counting up to 32% of the cases. Previously, we showed the potential of an implant made of poly(ε-caprolactone) (PCL) for AF defect closure. However, the slow in vivo degradation of PCL might hinder AF regeneration. Here, we hypothesized that accelerating PCL degradation kinetics by incorporating a lactide polymer known for its rapid degradation enhances AF regeneration.

Methods
A Poly(ε-caprolactone-co-lactide) (PCLA), was synthesized with various molar percentages of lactide (10, 20, and 30 %) and structures (copolymer or blend). These polymers were electrospun into sheets of aligned nanofibers. Then a multi-lamellar 3D implant was assembled by stacking the PCLA sheets and incorporating an anchoring system (wings that are glued on the adjacent vertebrae). The thermal, mechanical, and structural properties of the novel polymers were analyzed, including the impact of gamma-irradiation sterilization and their in vitro degradation over 6 months. Next, the cytocompatibility of the implant was assessed in vitro with ovine AF cells and ex vivo using a bovine tail disc model. Furthermore, the biomechanical performance of the implant was investigated using bovine tail motion segments (n=6 / group). Flexibility was tested under pure moments of +/- 1 Nm (1°/s) in all three motion planes and extrusion risk under dynamic loading (4 x 500 cycles) simulating typical daily activities. Finally, the implant's regenerative potential was assessed in a sheep model of simulated herniation (n=4).

Results
PCLA sheets had a Young's Modulus ranging from 20 to 37 MPa, close to that of AF tissue. Moreover, gamma irradiation (25 kGy) caused a Young's Modulus increase by 21-31 MPa when lactide > 20%. The 6-months in vitro degradation study of PCLA showed a molar mass loss of 70 ± 0.4% for the copolymer and 53 ± 17% for blends. Furthermore, scanning electron microscopy revealed fiber breakage in the copolymer. Furthermore, PCLA sheets guided the in vitro alignment and proliferation of ovine AF cells and maintained the expression of AF markers for up to 2 weeks (collagen type I and type II). Ex vivo, the multi-lamellar implant was maintained within a full-thickness annular defect (4mm biopsy punch) of bovine tail discs during 4 weeks of culture, and initial cell infiltration into the implant was observed. When additionally glued, the implant showed a reduced risk of extrusion compared to groups without gluing. The implant passed flexibility test but showed further need for improvement in the dynamic worst-case scenario. In vivo, despite instances of implant dislocation, disc hydration was preserved at one-month. Interestingly, substantial cellular infiltration and AF-like tissue ingrowth were evidenced within the implant layers.

Conclusions
This study confirmed the in vitro faster degradation kinetics of PCLA and the affinity of AF cells for the nanofibrous implant. It also highlights the ability of the newly designed multi-lamellar implant to instruct AF tissue regeneration. Future work will evaluate the long-term efficacy (6 months) in our sheep model.

Topic: Tissue-Specific Focus
Subtopic: Intervertebral disc & spine
Type: Oral presentation

TERMIS25_914 - Wnt-coated radiopaque hydrogels as an injectable and trackable bone filler material

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Introduction/Objectives
Degenerative bone diseases such as osteoporosis are debilitating for patients and costly to treat. The ability to efficiently engineer new bone tissue would be beneficial in this area, but remains a major challenge for regenerative medicine. Success in this area is dependent on incorporating clinically approved biomaterials that provide appropriate signalling cues to cells in order to control cell fate and direct tissue morphogenesis. Additionally, the ability to track biomaterials within bone using imaging modalities such as X-ray microtomography (μCT) is advantageous in orthopaedic surgery. Wnt signalling plays a leading role in regulating the stem cell niche in bone. The ability to direct new tissue formation by regulating osteogenic differentiation through Wnt functionalized materials is an attractive approach for treating a range of bone diseases. However, controlled manipulation of Wnt signalling for regenerative medicine has been limited due to concerns over unregulated activation of signalling. So the development of injectable materials, which are trackable *in vivo*, for controlled regulation of Wnt signalling provides a novel therapeutic route for engineering bone.

Methods
Wnt3a growth factor was conjugated to clinically approved polymers including Poly(ethylene glycol) diacrylate (PEGDA). The binding and retention of Wnt was tested using immunocytochemistry, ELISA and X-ray fluorescence (XRF). The signalling activity of the immobilised Wnt was confirmed using a T cell factor/lymphoid enhancer factor family (TCF/LEF) (Wnt pathway) reporter to directly monitor activation of the Wnt pathway. The ability of Wnt-functionalised PEGDA to support osteogenesis of mesenchymal stem cells (MSC) and patient derived bone cells was also determined by assessment of bone markers. The CT contrast agent Iohexol was also incorporated into PEGDA with its release monitored, and the radiopacity of the material was also determined using μCT.

Results
Results showed that Wnt3a can be immobilized and retained on the surface of synthetic polymer hydrogels. The biological activity of immobilised Wnt3a after coating on PEGDA was preserved as shown by TCF/LEF reporter activation. Wnt3a coated hydrogels supported osteogenesis of MSC cell lines as shown by positive expression of osteogenic markers. Whilst incorporation of Iohexol increased the radiopacity of the materials and was released over a 48h period.

Conclusions
Immobilised Wnt polymer materials such as PEGDA provide a new way of presenting growth factors for regulating cell behaviour and osteogenesis in an approach that can be used to spatially engineer the stem cell niche in a controllable manner. Immobilised Wnt polymers are amenable alongside cell therapies and can be combined with contrast agents allowing material tracking by CT. Ultimately, immobilised Wnt materials could form a platform technology for minimally invasive and injectable cell-based therapies to augment bone repair for the treatment of degenerative bone diseases such as osteoporosis.

Topic: Submit to SYMPOSIUM
Subtopic: Biomaterials design strategies for tissue engineering
Type: Oral presentation

TERMIS25_1249 - 3D printed inorganic/organic hybrids for large bone defect regeneration

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Introduction/Objectives
Bone can self-repair when defects arise due to trauma or disease, but beyond a particular size, it is irreparable without external intervention, so the need for novel materials to aid critical-size bone defect regeneration remains. Tissue engineering scaffolds show promise as they demonstrate bioactivity, compatible mechanical properties and suitable biodegradability. Here, we present novel, photocurable sol-gel Class II inorganic/organic (I/O) hybrid scaffolds, composed of Polycaprolactone (PCL) and silica, manufactured via Digital Light Processing (DLP).

Methods
The organic phase is composed of PCL-diacrylate combined with Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (TPO) photoinitiator; for DLP printing, a suitable photoabsorber is added. Tetraethyl orthosilicate (TEOS) is combined with 3-(Trimethoxysilyl)propyl methacrylate (TMSPMA), hydrochloric acid and distilled water to form the inorganic phase. Both phases are combined, and the hybrid resin is cured via UV light. Bulk samples were synthesised with varying post-synthesis conditions. Print resolution was optimised by photoabsorber and photoinitiator wt% alteration and geometries of increasing complexity were printed to achieve interconnected, porous structures. Fourier-Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA) were performed to identify functional groups, confirm successful synthesis of Class II hybrids and confirm I/O ratios. Uniaxial compression testing was performed to understand key characteristics, including Young's modulus and compressive strength. Dissolution studies were conducted for two sample groups in cell medium after sample incubation at various dilutions, and in distilled water for up to 7 days to understand ionic release. Cell studies were conducted with Saos-2 cells and hybrid dissolution products; fluorometric measurements were carried out at 3 timepoints to determine cell viability.

Results
Uniform bulk samples were successfully synthesised with FTIR spectra confirming formation of Class II hybrid networks, including sharp Si-O-Si Asymmetric Stretch bands at 1200-1000cm-1 demonstrating the formation of the inorganic silica network, and O-H Stretch bands at 3500-3200cm-1 indicating chemical bonding between both phases. Actual I/O ratios were calculated from TGA profiles and showed an average increase of +1.7% ±6% in mass compared to theoretical masses. Compression testing indicated maximum stress ranged from 62.3MPa±19.7MPa to 123.8MPa±3.7MPa and Young's Moduli of 145.8MPa±17.5MPa to 263.2MPa±9.8MPa across the distinct sample groups. Measurements for calcium, sodium, phosphorus and silicon from culture media dissolution products demonstrated no significant difference between sample groups, with average concentrations of 66.27ppm±0.25ppm, 1026.3ppm±4.57ppm, 32.06ppm±0.025ppm and 0.014ppm±0.005ppm respectively.

Conclusions
In this work, we successfully synthesised novel photocurable Class II hybrids consisting of a polymeric organic phase and silica inorganic phase, efficiently manufactured via DLP printing. Characterisation demonstrated consistent compositional profiles and promising mechanical properties, indicating great potential in future applications for large bone defect regeneration.

Topic: Submit to SYMPOSIUM
Subtopic: Cancer mechanobiology: factors to consider and exploit
Type: Oral presentation

TERMIS25_213 - Morphomechanic tuning of ERK by actin-TFII-I regulates cell identity

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Introduction/Objectives
Cell morphology is faithfully coupled to its identity, as abnormal morphology is the basis of cancer histopathology. While the cell form-identity relationship holds true, how these two parameters are mechanistically coupled remains unclear. Furthermore, specific cell morphology is often seen as the product of gene expression programs, passively serving as an indicator of cell identity. However, it is possible that cell morphology could play a more active role in setting cell identity. Insights into these questions could help the rational design of cell fate engineering and targeting approaches.

Cell morphology has been mostly examined in the x-y dimension, e.g. by patterning the size/shape of the surface area on which cells adhere and grow, or by physically stretching the surface that cells

grow on. The significance of cell's z dimension has only begun to be appreciated when cell height confining devices were implemented, although these efforts focused on short time scales (minutes) insufficient for most cell fate changes. Recent years have also witnessed the explosion of 3D culture techniques (e.g. spheroids, organoids, embryoids and more) that capture cell fates far beyond those attainable from the same cells in 2D cultures, suggesting the possibility that cells' z-dimension could offer one of the distinguishing cues for emerging cell identity. As most 3D culture models involve complex biochemical and biophysical cues, the contribution by cells' z-dimension to cell identity remains challenging to define.

Methods
We defined the molecular players and their modes of action in sensing and regulating cells' z-dimension by leveraging somatic cell reprogramming into pluripotency, a process that follows choreographed cell morphological changes. Specifically, reprogramming with the Yamanaka factors begins with somatic cells grown in 2D, and end as pluripotent cells in *colonies*. The colony morphology of established pluripotent stem cells (induced pluripotent stem cells, iPSCs; embryonic stem cells, ESCs) is well appreciated and informs the day-to-day assessment of the culture quality: naïve iPSC/ESC colonies are "dome-shaped" and the loss of this domed morphology indicates exit from naïve pluripotency. We measured and purturbed the cell/colony height and assessed the functional consequences in cell identity.

Results
Pluripotent cells and somatic cells reprogramming into pluripotency allocate large amounts of actin into their nucleus, which morphs cells to become taller than 10 μm, a minimal height required for the pluripotent identity. Accumulated nuclear actin binds to TFII-IΔ, an atypical transcription factor that translocates into the nucleus upon signaling. TFII-IΔ also binds to and activates ERK. The binding of TFII-IΔ by nuclear actin reduces ERK activity, in coordination with changes in cell/colony height. The tight coupling between cell height and nuclear actin accumulation necessitates the degree of ERK tuning to be mild. Mild ERK inhibition by chemicals recapitulates the tuning by actin-TFII-IΔ and turns most cells in reprogramming cultures into pluripotency. Thus, morphomechanic fine tuning of ERK activity level imparts a cell fate that is otherwise extremely rare.

Conclusions
We uncover a novel mechanism for how cell morphology couples to its identity via the actin-TFII-IΔ-ERK axis, identifying points of intervention in cell fate manipulation.

Topic: Cancer
Subtopic: Tumor microenvironment
Type: Oral presentation

TERMIS25_773 - DIGITAL FUNCTIONAL IMAGING AT MICROSCALE: A STUDY OF OSTEOSARCOMA TREATMENT RESISTANCE

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Introduction/Objectives
Osteosarcoma is a malignant primary bone tumor affecting children and young adults and characterized by bone anarchic production by tumor cells. Recurrent therapeutic molecular targets or specific treatment resistance bio-markers have not found yet because of high genomic complexity which leads to intra-tumoral and inter individual heterogeneity [1]. Metabolomic, molecular, tissue and macroscopic scales are studied. It appears that mechanical stimuli plays a significant role on tumor microenvironment while increasing complexity. We hypothesized that tumoral tissue mechanobiology might be investigated a multiscale approach involving heterogeneous porous media with fluid, cells, fibrous and mineralized or solid phases. The methodology was guided by clinical routine histological images to explore treatment resistance.

Methods
Correlations between tissue porosity and microenvironment cell density [2] were explored using upscaling and machine learning methods adapted to large images, namely 50000px * 50000 px from histological and immunohistological sections. A dedicated image segmentation process preceded an innovative digital functional imaging technique based on porous media theory combined with a sequential grid block approach [3]. Targeted cells were macrophages and lymphocytes. Tissue mechanical properties and interstitial fluid transport were investigated. It concerned piece-wise constant equivalent parameters such as tissue porosity and permeability, stiffness coefficients and fluid velocity maps. Image segmentation gave access to cell phases distribution. This deterministic quantification faced clinical records and dedicated correlation statistical methods were implemented.

Results
We identified that a negative correlation between bony matrix porosity and macrophage density was a marker of good response of osteosarcoma patients to chemotherapy. Moreover, relative high lymphocyte density was found to be a marker of patient survival without metastasis development or relapse. We found correlations between tissue stiffness properties, transport properties and cell distribution into the microenvironment depending upon treatment response and we highlighted the impact on neo-formed bone microarchitecture. It appeared that chemotherapy was more efficient in lacy-like neo-formed bone regions compared to trabecular-like neoformed bone regions. Zones of good response were matching with a decreasing number of residual cells and decreasing values of tissue permeability and stiffness.

Conclusions
To conclude, we have developed an innovative approach based on heterogeneous porous media theory and based on data from clinical setting to explore osteosarcoma microenvironment properties and response to treatment. Currently investigated on a large cohort, those new identified mechanobiological responses and pronostic signatures could be integrated into data stratification algorithm to improve patient specific therapies. Furthermore, this innovative in silico functional imaging technique is versatile and can be readily applied to enhance treatment for other cancers.

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Topic: Submit to SYMPOSIUM
Subtopic: Cancer mechanobiology: factors to consider and exploit
Type: Oral presentation

TERMIS25_1284 - Molecular extensibility as a biophysical cue for disseminated cancer cells

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Introduction/Objectives
Mechanical properties of the tumor microenvironment and associated biochemical signaling play an important role in tumor progression and metastasis. Still, it is unclear to what extent they regulate the fate of disseminated cancer cells–their survival, dormancy, or reawakening. The presented study aims to investigate the role of the biophysical properties of the environment on metastatic cancer cells by examining the influence of molecular extensibility of the extracellular matrix on cellular responses.

Methods
We prepared and characterized surface coatings based on tropoelastin and poly (hydroxyethyl methacrylate) (pHEMA). The latter was also optimized to enable high throughput for cell biological experiments by immobilizing pHEMA block copolymers on polystyrene, followed by biofunctionalization with GRGDS as an important

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integrin-binding peptide. The pHEMA-based model system offers varying molecular extensibilities depending on the molecular weight of pHEMA-block-copolymers. We then investigated cell responses dependent on varying molecular extensibilities via immunofluorescence, label-free 3D live cell imaging, single-cell force spectroscopy, and cell proliferation studies.

Results
Different morphologies and cell-matrix adhesion responses of cancer cells were observed on the highly extensible biomolecule tropoelastin, compared to cross-linked tropoelastin with reduced extensibility. In addition to tropoelastin, with multiple cell adhesion motifs, we developed a synthetic, biomimetic, and extendable polymer model system based on pHEMA for mechanosensitivity studies - allowing us to study the influence of extensibility decoupled from biochemical cues. The biofunctionalization of the pHEMA-coated surfaces with GRGDS peptides was validated using cleavable fluorescently labeled GRGDS peptides and progressive stages of biofunctionalization were followed with quartz crystal microbalance with dissipation (QCM-D). Metastatic cancer cells seeded on varying molecular weight pHEMA-coated surfaces exhibited different morphologies depending on the molecular extensibilities. We observed that MDA-MB-231 cells adhered more strongly to lower molecular weight pHEMA. To better understand the mechanisms leading to distinctive cellular responses on different molecular weight pHEMA-coated surfaces, we did a proteomic analysis of adsorbed proteins from cell-culture media on biofunctionalized pHEMA-coated surfaces and observed that adsorbed proteins differ depending on the molecular weight of pHEMA. We are currently testing proliferation and dormancy markers and live cell imaging with cell cycle indicator cell lines on both tropoelastin and pHEMA-based model systems to investigate if molecular extensibilities also potentially influence tumor cell cycling, proliferation, and dormancy.

Conclusions
The results obtained will help us to establish pHEMA as a model system for mechanosensitivity studies for metastatic cancer cells and enhance our understanding of the role of molecular extensibility as a biophysical cue in regulating the fate of disseminated tumor cells.

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_145 - Biologically-inspired melt electrowriting for the generation of highly biomimetic functional and therapeutic myocardium

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Introduction/Objectives
In the heart, the specific 3D structure produces an efficient ejection of blood, through the contribution of myocardial layers contracting in-plane. When myocardial infarction strikes, this architecture is disrupted, adding a disarranged contraction to the decreased availability of pumping units (cardiomyocytes). Melt electrowriting (MEW) is a high precision additive manufacturing technology, with capacity to direct cellular alignment. In this work, we characterize the alignment of cardiac fibers in a large animal model (pig) and MEW to fabricate a bio-inspired scaffold.

Methods
A design with pores in the shape of diamonds was hypothesized to be able to generate a cellular alignment resulting in a cardio-mimetic in-plane contraction. This was printed with MEW in medical-grade poly-ε-caprolactone. Human induced pluripotent stem cells (hiPSCs) were differentiated to cardiomyocytes (CMs) and cardiac fibroblasts (CFs), mixed in a 9:1 proportion, embedded in a fibrin hydrogel and casted around the diamond-pore MEW structures. Cell viability, metabolic capacity, gene expression (by qPCR and bulk RNAseq), functionality (contraction kinetics, optical mapping) and structure (confocal microscopy), in comparison with orthogonal (square and rectangular) designs. Finally, engineered cardiac tissues were tested for their potential therapeutic capacity in an athymic rat model of myocardial infarction.

Results
The generated MEW-diamond tissues showed in-plane macroscopic contraction for over 1 month, with significantly faster kinetics, increased force and higher conduction velocity than those based on square or rectangular pores. Our diamond design induced a specific hiPSC-CM alignment resulting in the observed in-plane contraction. Transcriptomic analysis using bulk RNA-seq reveals diamond-MEW tissues present features of maturation as compared to traditional 2D cultures, with an upregulation of sarcomeric and lipid and potassium metabolism genes. Finally, we employ the bio-inspired MEW cardiac tissues to treat a myocardial infarction model in athymic rats. Treated animals show a significant improvement in systolic contractility and ventricular remodeling, which is absent in the control group. This is tied to the presence of large grafts of human cardiac cells, remuscularizing the ventricular wall.

Conclusions
In this work, we translate the natural myocardial structure and function to a MEW scaffold design able to deliver a superior and more mimetic functionality. The human engineered tissues show features of maturation, and are able to provide support to an injured heart in vivo. This work sets the foundations for the translational advancement of MEW-based structures, by conducting the first in vivo assessment in a model of cardiac disease.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_700 - Optimized 3D bioprinted atrial tissue model for studying atrial fibrillation and fibrosis

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Introduction/Objectives
Atrial fibrillation (AF) poses serious health risks, including stroke, yet current models lack the complexity needed to study it effectively. This study aims to develop 3D bioprinted fibrotic atrial tissue models using human induced pluripotent stem cell-derived atrial cardiomyocytes (hiPSC-aCMs) and cardiac fibroblasts (hiPSC-CFs) to better replicate AF mechanisms and support therapeutic advancements.

Methods
This study comprises three primary components: (1) Bioink optimization of gelatin methacryloyl (GelMA) with rheological properties tailored to support hiPSC-aCM and hiPSC-CF viability and function. Rheological properties, including storage modulus (G') and loss modulus (G''), were assessed to determine the bioink's structural stability. (2) Bioprinting of atrial tissues using two approaches: a mixed approach where hiPSC-aCMs and hiPSC-CFs are combined in a single bioink, creating a homogeneous cellular environment, and a patterned approach that places hiPSC-aCMs and hiPSC-CFs in

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defined regions to replicate the spatial organization of native atrial tissue. (3) Functional analyses such as optical mapping to assess the tissue's electrophysiological response, including action potential duration, and tolerance to stimulation frequencies.

Results
The optimized GelMA-based bioink showed strong rheological stability, with a storage modulus of approximately 30 kPa, making it ideal for bioprinting. Using 10% GelMA concentration, 80 kPa printing pressure, 3 mm/s speed, 22°C, an 80-second crosslinking time to 405 nm light, and a 22G nozzle, the bioink achieved the right balance of structural integrity and cell compatibility.

Bioprinting using the mixed approach resulted in a homogenous distribution of hiPSC-aCMs and hiPSC-CFs and supported high cell viability and functional connectivity. In the bioprinted constructs, sarcomere alignment achieved an Orientation Parameter P (OPP) of 78%, indicating a high degree of structural maturity necessary for effective cardiac contraction. Immunocytochemistry analyses revealed strong expression of connexin 40 and connexin 43, suggesting the potential for connectivity between cardiomyocytes (CMs) and cardiac fibroblasts (CFs) within the tissue. This connectivity between hiPSC-aCM and hiPSC-CFs may play a role in enhancing the maturation of cardiomyocytes through possible gap junction formation.

Optical mapping demonstrated that the bioprinted constructs could tolerate stimulation frequencies up to 4 Hz, confirming their electrophysiological robustness. APD_{90} measurements indicated a value of 250 ms, which is consistent with atrial, rather than ventricular, cell characteristics, supporting the model's relevance for studying functional electrophysiological aspects of AF.

Conclusions
This research successfully established a 3D bioprinted atrial tissue model with optimized bioink properties and physiological relevance, facilitating advanced AF and fibrosis studies. Bioprinting approaches allowed for flexible modeling of cellular organization. Sarcomere alignment, cellular connectivity, and electrophysiological properties met essential requirements for a robust in vitro model. This model provides a valuable platform for exploring AF mechanisms and testing anti-fibrotic therapies, offering a significant advancement in cardiac tissue engineering.

Topic: Immune System & Inflammation
Subtopic: Immune cells
Type: Oral presentation

TERMIS25_980 - Development of humanized, functional, tissue engineered in vitro model of healthy myocardium

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Introduction/Objectives
The myocardium is a contractile tissue that hosts multiple cell types that work in unison to maintain a steady supply of oxygen and nutrients throughout the body. Furthermore, research over the past decade has significantly improved our understanding of the interaction between cardiomyocytes and immune cells, illuminating the importance of cardiac resident macrophages in maintaining homeostasis within the myocardium. In particular, macrophages in the heart perform a number of critical functions such as facilitating electrical conduction, capture and elimination of cardiac exophers and routine immunosurveillance[1]. Here, **generate a tissue engineered model of healthy myocardium**, with an innate immune response capability for patient-specific drug screening, regenerative medicine and drug discovery applications.

Methods
iPSCs were expanded and differentiated to obtain macrophages (iMacs) and cardiomyocytes (iCMs). To understand the effect of iCM secretome on the polarization and maturity of iMacs, iMacs were first cultured in iCM conditioned media, followed by a co-culture with iCMs. The phenotypic and functional capabilities of iMacs were assessed using flow cytometry and qRT-PCR. Similarly, the maturity of iCMs in the presence of iMacs was studied using qRT-PCR and immunofluorescence imaging. Next, an electrical stimulation (ES) regime was developed to facilitate iCM maturation. Finally, a tissue engineered heart tissue (EHT) model was developed using type I collagen and Matrigel^{TM} to fabricate 3D models of the myocardium.

Results
The iMacs that were generated were found to be $CD14^{high}CD11b^{high}$ and $CX3CR1^{high}HLA\text{-}DR^{high}CCR2^{low}$. Additionally, iMacs were able to perform phagocytosis as well as polarize to become classically and alternatively activated macrophages. The iCMs generated were $cTnT^{high}MLC2a^{high}$ and performed spontaneous beating from day 7 of differentiation. Furthermore, iCM conditioned media was found to drive a pro-regenerative response in iMacs while iMacs in co-culture with iCMs maintained a quiescent phenotype, similar to control conditions. Finally, the ES regime used was found to support robust maturation of iCMs, with an upregulation in genes MY6, MY7, SERCA2 and GJA1.

Conclusions
Firstly, we demonstrated the generation and characterisation of iMacs and iCMs from the same iPSC parent line. Next, we showed the functional differences in iMacs when cultured with iCM conditioned media as well as in co-culture with iCMs. Finally, we demonstrated the successful maturation of iCMs under an optimized ES regime. Future work includes the assessment of the EHTs and understanding the effects of iMacs in improving the maturity of the engineered cardiac tissue.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_985 - Bioengineered biphasic cardiac models for cardiotoxicity assessment

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Introduction/Objectives
Humans are continuously exposed to toxic substances, and cardiotoxicity is still poorly investigated, although several chemicals have shown cardiotoxic effects. Furthermore, age-related changes in myocardial structure and function are responsible for changes in tissue response to chemical exposure. In this context, precise and faithful models replicating different myocardial aging stages are needed not only to better understand physio-pathological processes and more accurately assess the effects of therapies and chemicals, but also to

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replace animal testing. In this work, we developed 3D cardiac models mimicking different myocardial aging stages (i.e., young and mature tissue) and evaluated their potential for chemical cardiotoxicity assessment.

Methods
Models consisted of a fibronectin (FN) functionalized scaffold produced by melt extrusion additive manufacturing of a custom-made poly(caprolactone)-based polyurethane (PUR) and a photocurable Gelatin-Methacryloyl (GelMA) hydrogel at 5% and 10% w/v concentration to replicate young and mature myocardium, respectively. GelMA gels acted as a carrier for a co-culture of human induced pluripotent stem cell-derived cardiomyocytes and human coronary artery endothelial cells (80:20 ratio). The bioengineered models were assembled by pipetting the cellularized GelMA solution into the PUR scaffolds, followed by photo-crosslinking at 365 nm and 10 mW/cm2 for 30s. The models were validated with doxorubicin and used to test the toxicity induced by different chemicals (e.g., rotenone).

Results
Infrared spectroscopy and size exclusion chromatography proved PUR successful synthesis, while thermal and rheological tests provided insights into its thermal transition, stability, and thermomechanical behavior. Scaffolds were then produced with an anisotropic geometry (filament ca. 0.41 mm) and surface functionalized with FN for more realistic mimesis of cardiac tissue. GelMA hydrogels at 5 and 10% w/v were characterized by photo-rheology evidencing Young's modulus (10 vs. 55 kPa) replicating young and mature cardiac tissue, respectively. Upon assembly, scanning electron microscopy images of the biphasic structures evidenced a good PUR-GelMA interaction and interconnected pores favoring cell proliferation by allowing nutrient and oxygen diffusion, and waste removal. Cell-based models were cultured for up to 11 days, and cell viability and oxidative stress were monitored over time in control and chemically treated systems. Multi-omics analysis and integration (including transcriptomics, proteomics and metabolomics) showed that myocardial aging increased specific biomarkers related to a more pronounced mitochondrial dysfunction, protein breakdown, and amino acids and lipid profile impairment, affecting muscle contraction and cell maturation. Chemicals further modulated the observed pathways in control cells, focusing on those related to cell death and metabolic burden in particular.

Conclusions
Overall, this work demonstrated that the developed 3D models can effectively recapitulate in vitro mature and young cardiac tissue and represent promising tools for cardiotoxicity testing.

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Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1161 - The combination of passive and active mechanical loadings improves contractility and maturation of three-dimensional engineered cardiac tissues

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Introduction/Objectives
The development of functional three-dimensional engineered cardiac tissue (ECT) models is essential for advancing the understanding of cardiac diseases and potential regeneration mechanisms. Physiological cues, resembling those of native myocardium, enhance cardiac maturation and functionality in vitro. However, current bioreactors often provide limited physical stimulation options. To address this, a millimetric-scale microscope-integrated bioreactor was developed to deliver passive and active mechanical loading, along with electrical stimulation, to ECTs. We previously demonstrated that ECTs subjected to passive stimulation exhibited superior functionality, sarcomere organization, and increased cell elongation compared to static controls during an 8-day culture period. However, cell elongation and maturation were predominantly observed at one edge of the ECT.

This study aims to investigate whether applying passive mechanical loading followed by additional active cyclic mechanical loading could further enhance ECT functionality and spatial uniformity of cardiac maturation within an 8-day timeframe.

Methods
ECTs were generated by seeding either neonatal rat cardiomyocytes (NRCM, 70%) and neonatal rat fibroblasts (NRFB, 30%) or human induced pluripotent stem cell derived cardiomyocyte (hiPSC-CM) in a fibrin gel solution (15 mg/mL fibrinogen, 5 U/mL thrombin, cell-density: $20x10^6$ cells/mL). Passive loading (PL) was applied for 8 days, either alone or in combination with active cyclic loading (AL) during the final 4 days of culture. ECT functionality was evaluated by measuring Excitation Threshold (ET) (minimum electrical field at which ECT starts beating synchronously), Maximum Capture Rate (MCR) (maximum value of frequency that the ECT can follow), and force of contraction. Additionally, the response of ECTs to varying calcium concentrations was assessed by measuring changes in contraction force. Immunofluorescence staining for cardiac markers was performed to assess cardiac maturation.

Results
In ECTs composed of NRCMs and NRFBs, the combination of PL+AL enhanced tissue functionality compared to PL alone, as indicated by improvements in ET (PL: 5.64±4.40 V/cm vs. PL+AL: 4.83±1.37 V/cm) and MCR (PL: 2.66±1.66 Hz vs. PL+AL: 3.22±1.09 Hz, $p>0.5$), as well as a significant increase in developed force (PL: 0.05±0.03 mN vs. PL+AL: 0.25±0.20 mN, $p<0.0001$). Similar trends were observed with hiPSC-CM-derived ECTs, where PL+AL improved ET (PL: 6.01±4.96 V/cm vs. PL+AL: 6.69±5.27 V/cm, $p>0.5$), MCR (PL: 1.71±0.47 Hz vs. PL+AL: 2.15±0.55 Hz, $p<0.5$), and developed force (PL: 0.12±0.06 mN vs. PL+AL: 0.13±0.05 mN, $p>0.5$). When exposed to increasing calcium concentrations (starting at 0.3 mM), all groups showed an enhancement in contraction force, peaking at 10 mM. However, calcium sensitivity differed between groups. ECTs subjected to PL+AL exhibited higher sensitivity to external calcium compared to those exposed to PL alone.

Cell elongation and cardiac maturation (based on sarcomere organization) appeared improved and more uniform throughout the construct, in both NRCM+NRFB and hiPSC-CM groups, compared to ECTs exposed only to PL.

Conclusions
The combination of passive and active mechanical loading improved cardiac maturation and elongation throughout the whole ECT and increased the force of contraction in just 8 days of culture compared to the application of passive loading alone using both rat cells and hiPSC-CM.

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1493 - Human induced pluripotent stem cell-derived engineered heart tissue model of cardiac fibrosis for medium-throughput functional screening

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Introduction/Objectives
Cardiac fibrosis is a serious complication of a majority of cardiovascular pathologies. Unchecked stiffening of the myocardium upon fibroblast activation and subsequent over-deposition of extracellular matrix (ECM) components leads to impaired relaxation capabilities, arrhythmias, hemodynamic disturbances, and ultimately heart failure. Despite these serious implications, the pathophysiology of the disease is not sufficiently understood and the available treatment options targeting excessive scar formation are limited. Therefore, the project aims to develop human induced pluripotent stem cell (hiPSC) - derived 3D engineered heart tissue (EHT) models of cardiac fibrosis to investigate the underlying pathomechanisms of the disease and explore preventative or therapeutic interventions.

Methods
Cardiomyocytes were differentiated from hiPSCs as previously described and their purity was assessed via flow cytometry staining. For the differentiation of cardiac fibroblasts, hiPSC-derived epicardial cells were directed toward a mesenchymal fate. The obtained stromal cells were characterised via flow cytometry, immunocytochemistry stainings, and RNA sequencing. Subsequently, the cardiac resident cells were combined in a collagen-based scaffold and subjected to maturation through mechanical stimulation over the course of 21 days. The functional properties of EHTs were assessed via a custom-made bioreactor system. Increasing ratios of cardiac fibroblasts to cardiomyocytes within the EHTs were tested, along with additional stimulation with fibrosis-inducing transforming growth factor β (TGF-β) to procure a fibrotic response. The stiffness of the tissue constructs was analysed via nanoindentation and excessive ECM deposition was verified with immunohistochemistry stainings.

Results
We were able to reproduce an in vitro fibrotic phenotype within hiPSC-derived engineered heart tissues in regard to their functional and structural characteristics. The functionality of the fibrotic tissues evaluated by spontaneous and electrically stimulated contractile forces decreased significantly in comparison to control EHTs. Furthermore, an in-depth analysis of contraction kinetics revealed a pathological response to stimulation with a sympathomimetic drug, isoproterenol, suggesting a distorted signal propagation in fibrotic constructs containing the highest fibroblast content, congruent with a stiffened myocardium. Amplified ECM deposition in fibrotic bioartificial tissues was confirmed by immunohistochemistry stainings. Subsequently, to increase the possible throughput offered by the established engineered heart tissue model of cardiac fibrosis, a new bioreactor system was developed and validated.

Conclusions
Here we report on the establishment of a 3D-engineered heart tissue model of cardiac fibrosis utilising iPSC-derived cardiac resident cell types. To make the disease model suitable for screening purposes, the established EHTs were downscaled and a new mechanical bioreactor system was designed. The system offers a straightforward and rapid on-demand readout. Equipped with the described EHT model of fibrosis and the possibility of functional and structural analysis in the medium throughput range, we aim to improve the understanding and potential management of the disease.

Topic: Cells
Subtopic: Control of phenotype & function
Type: Oral presentation

TERMIS25_398 - Chemically programmed metabolism drives a superior cell fitness for cartilage regeneration

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Introduction/Objectives
Cell therapies hold the promise to treat various human diseases, attracting much attention on fundamental cell features in establishing functional cell implants. Cell fitness, is one of the most vital properties that endow cell graft with a good adaptation to changeable environments. Only fitter (winner) cells are estimated to thrive, while less-fit (loser) cells will be eliminated. Adult (mature) chondrocytes are typically less-fit cells with poor self-regenerative properties in cartilage degeneration, and their poor fitness also compromises the outcomes of chondrocyte implantation for cartilage injury treatment. Concerning that fitness regulatory genetic signals are deeply involved in oncogenic pathways, there is an urgent need to develop safe and cost-effective therapeutics to manipulate human cell fitness for promoting tissue repair.

Methods
Small molecular chemical compounds can serve as powerful tools to directly control proteins in a fine-tuning manner without inducing oncogenic genes. They show distinct advantages in manufacture, storage, transportation and standardization. In this study, we identified fitness regulatory small molecule compounds based on our previously established drug screen model, which reflected a two-staged chondrocytes' fitness transition. In a chemical library containing over 2000 molecules, we used high-content imaging to identify candidate drugs that significantly promoted chondrocyte fitness and employed multiple tests to analyze the candidate's function and the mechanisms of its action.

Results
We achieved a cost-effective manipulation of human cell fitness using a single chemical compound FPH2, to promote articular cartilage repair. FPH2 significantly improved human chondrocytes' performance in multiple tests, which showed a stronger ability to proliferate, migrate and maintain chondrocyte functionality. Single-cell transcriptomics revealed that FPH2 induced a winner status highly expressing abundant fitness-responsible genes associated with metabolism, protein translation, migration and self-protection. The mechanism was primarily based on a modified metabolic pattern with the inhibition of carnitine palmitoyl transferase I, which induced beneficial metabolites' accumulation, boosted energy production and maintained mitochondrial homeostasis. In animal studies, FPH2-treated human adult chondrocytes demonstrated a superior hyaline cartilage regeneration with improved tissue interface healing, in a rodent full-thickness cartilage defect model. In another partial-thickness model, the FPH2-loaded hydrogel also protected joint surfaces from developing early osteoarthritis, highlighting its potential to modulate tissue niche.

Conclusions
This proof-of-concept study demonstrated the possibility of manipulating cell fitness by a single chemical molecule, which provides a handy and powerful tool to be adopted in cell culture and tissue regeneration. These findings also introduce a new chemically programmed cell status to update the concept of cell fitness, and provide mechanistic insights into the underpinning molecular machinery.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_580 - 3D Bioprinting of meniscus derived microtissues to direct collagen organisation and engineer functional meniscal grafts

Kaoutar Chattahy, Gabriela S. Kronemberger, Aliaa Karam, Daniel J. Kelly

Introduction/Objectives
Meniscus injuries affect over 1.5 million individuals annually worldwide. Common meniscal injury repair interventions alter joint biomechanics and significantly increase the risk of developing osteoarthritis. Meniscus regeneration remains a great challenge due to their poor intrinsic healing potential, resulting in joint degeneration. This drives the need for effective meniscal tissue engineering solutions to reconstruct the structural inhomogeneity and anisotropy of meniscus tissue, including its zonal composition, structure and biomechanics, particularly the circumferentially aligned collagen fibers that provide tensile strength and stiffness. One promising

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approach in tissue engineering is 3D bioprinting, which offers an ideal route for the precise deposition and patterning of cells, growth factors and supporting materials with high spatial resolution. In this study, we aim to 3D bioprint biomimetic scaffold-free meniscal grafts by leveraging the capacity of meniscal progenitor cell-derived microtissues to self-organize into complex anisotropic meniscal fibrocartilage tissue. Our approach uses a bioink composed of microtissues at a very high density, to enable the engineering of mechanically functional and structurally organised meniscal grafts.

Methods
Various densities of meniscus progenitor cell (MPCs) derived microtissues (20,000, 30,000, and 45,000 microtissues/ml of bioink) were combined with 1% gelatin to prepare the bioink. The bioink was loaded into a 3 mL syringe and placed in the syringe pump printhead of the Cellink BioX6. Following calibration and extrusion tests, we began bioprinting with an extrusion rate of 6 μL/s and a speed of 3 mm/s onto a methacrylated xanthan gum support bath. Post-bioprinting, the support bath was crosslinked using UV light and the entire construct was cultured in chondrogenic media for 4 weeks. We initially bioprinted straight lines and circumferential patterns, then optimized the spacing by bioprinting lines at varying distances (2 mm and down to 0.25 mm). The bioprints were assessed biochemically and histologically, with collagen fiber orientation analyzed using polarized light microscopy.

Results
The bioink with 45,000 MPC-derived microtissues/ml produced the most continuous, robust tissue filaments, forming well-fused structures that could be lifted from the methacrylated bath intact. Tissue generated using the higher 45,000 microtissues/ml bioink density also stained more intensely for sGAG and collagen, particularly type I collagen deposition. These constructs also stained positive for alizarin red, indicating early mineral deposition. Organized collagen fibers were observed in both the 30,000 and 45,000 microtissues/ml groups. Optimal print fidelity was achieved in a 1% XG-MA support bath, with enhanced resolution achieved using a 22G needle. This support bath also enabled microtissue laden filaments to be deposited side-by-side, thereby enabling the 3D bioprinting of complex structures.

Conclusions
Gelatin based bioinks containing high microtissue densities support robust microtissue fusion, robust ECM deposition and the development of robust and organized fibrocartilage filaments, highlighting their potential for developing robust meniscal grafts. These findings highlight the potential of high microtissue density bioinks for engineering structurally organised, mechanically functional, and clinically relevant meniscal implants.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

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TERMIS25_788 - Guiding Cells Under Pressure: Pneumatic Bioprinting for Spatially Organized Cartilage Tissue Fabrication

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Introduction/Objective
Cartilage defects caused by trauma or osteoarthritis significantly reduce patients' quality of life. Recreating the zonally distinct structure of cartilage tissue, essential for its shock-absorbing function, remains a major challenge. This study explores how varying pneumatic pressures before bioprinting influence the cartilage-forming capacity of human nasal septum chondrocytes (hNCs).

While recent advances in bioink formulations have enabled spatially organized tissues through strategic bioink combinations, our approach simplifies this process by using a single bioink composition across all layers and priming hNCs with pneumatic pressure before extrusion. Chondrocytes are highly responsive to mechanical stimuli, adapting their morphology and function based on external forces. Utilizing this key aspect of extrusion-based 3D bioprinting offers exciting possibilities for engineering functional cartilage tissue and enhances clinical translatability by avoiding the complexities of multiple bioink formulations.

Methods
Human NCs isolated from nasal septal cartilage biopsies (N=3) were expanded up to P3. Hyaluronic acid (1.64MDa) functionalized with tyramine (THA) was combined with collagen fibrils and enzymatically crosslinked using horseradish peroxidase and hydrogen peroxide. The bulk hydrogel was mechanically fragmented via extrusion through a cell strainer (5μm). The resulting granular gel was supplemented with a solution of low molecular weight (280kDa) THA, hNCs (20×10^6 cells/mL), and a photoinitiator system based on ruthenium/sodium persulfate (0.2 mM/0.5 mM).

The prepared bioink containing the hNCs was loaded into a cartridge and connected to the pneumatic printing unit of the 3D bioprinter (RegenHU, Switzerland). The bioink containing the cells was exposed to 4.5 bar pneumatic pressure for 30 minutes. The control group was also loaded into the cartridge, but no pressure was applied. The bioink was transferred into cylindrical molds and crosslinked using blue light (450 nm, 5 mW/cm^2, 40 seconds). The constructs were cultured in chondrogenic medium (DMEM-HG, 5% FBS, insulin 40 μg/mL, ascorbic acid 0.1 mM, and BMP-2 100 ng/mL) for 28 days. Viability assessments using calcein-AM/ethidium-homodimer-1 and Hoechst 33342 were performed on days 1, 3, and 7. The effects of pressure on cell morphology and tissue formation are being evaluated histologically (Saf-O, col II staining), biochemically (GAG and DNA) and by RT-PCR.

Results
Cell viability was preserved in both groups and remained stable throughout the 28-day culture period. Safranin-O staining showed that pressure-exposed cells adopted a fibroblastic morphology with limited glycosaminoglycan (GAG) accumulation. In contrast, control group hNCs exhibited a round morphology, formed clusters, and produced an abundant GAG-positive matrix. Biochemical and RT-PCR analyses of the samples are currently ongoing.

Conclusions
This study highlights the potential of pneumatic 3D bioprinting to enhance the cartilage-forming capacity of hNCs. Fine-tuning the pneumatic pressure before extrusion could enable the fabrication of spatially organized cartilage tissue that mimics the layered structure of native cartilage, offering a promising approach for tissue engineering applications.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_810 - 3D bioprinting of articular cartilage progenitor cell derived microtissues within a physically constraining support bath to engineer structurally organised cartilage

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Introduction/Objectives
Engineering functional articular cartilage (AC) is a significant challenge in tissue engineering. Recapitulating the arcade-like collagen organization in native AC, crucial for its strength and stiffness, is key to developing functional grafts. This highlights the need for novel strategies to control collagen alignment in engineered tissues. Therefore, in this study, embedded bioprinting techniques were used to impose spatially defined boundary conditions on AC progenitor cell (ACP) derived microtissues, guiding their fusion and

extracellular matrix (ECM) secretion to form anisotropic AC structures. To achieve this, we first examined how external hydrogel boundaries of varying widths influence neotissue organization of ACP microtissues. Next, we employed a support bath that enabled high-resolution bioprinting and functioned as an external boundary to direct anisotropic collagen alignment following the deposition of ACP derived microtissues.

Methods
ACPs were isolated from goat AC through differential adhesion to fibronectin. Positive molds with cuboidal channels (12mm long, 1mm high, and widths of 250, 500, or 750μm) were printed using a stereolithography printer (Formlabs, Massachusetts, USA) and used to cast channels in agarose (4% w/v). ACP microtissues (1,000 cells/microtissue) were cast into the agarose channels. Methacrylated xanthan gum (XGMA) was prepared by dissolving xanthan gum (0.5% w/v) in deionized water followed by adding glycidyl methacrylate (7.41% v/v), and stirring overnight at 60°C. The solution was dialyzed (MWCO 6–8 kDa) and freeze-dried. The bioink was prepared by combining 45,000 ACP microtissues with 1mL 1% (w/v) gelatin. Next the bioink was bioprinted in a 1% (w/v) XGMA support bath using a 22G needle on the CELLINK BIOX6 (Gothenburg, Sweden) into a sheet-like structure composed of adjacent lines of 250μm wide filaments. In all experiments chondrogenesis was assessed after four weeks of culture through histology, immunohistochemistry, and biochemical assays.

Results
All channel widths supported robust chondrogenesis as demonstrated by positive staining for glycosaminoglycans and collagen. Polarized light microscopy revealed that collagen fibers aligned parallel to the agarose boundary edges in the 500 and 750μm channels. In the 250μm channel, a more organized collagen structure was observed throughout the depth of the filament, with fibers aligned parallel to the channel's long axis. This was also reflected in the directionality analysis where the 250μm channel had a significantly higher coherency indicating a more aligned collagen network. Similarly, filaments in the bioprinted constructs aligned along the printed sheet's long axis, with greater microtissue fusion compared to the casted 250μm channels.

Conclusions
External hydrogel boundaries can effectively control collagen alignment in engineered tissues generated using ACP derived microtissues. Increased collagen alignment was observed in the thinner (250μm) casted filaments. Bioprinted ACP microtissues fused and secreted a collagen rich ECM aligned along the long axis of the printed construct forming an anisotropic tissue. Future work on bioprinting functional AC will utilize the XGMA bath to provide horizontal and vertical guiding structures in the superficial and middle/deep zones of the construct to achieve an arcade-like collagen architecture.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

TERMIS25_820 - Regional transcriptional and proteomic characterisation of human meniscus informs parameters for generation of 3D bioprinted meniscus analogues

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Introduction/Objectives
Meniscal injuries affect over 1.5 million people across Europe and the USA annually. Injury greatly reduces knee joint mobility and quality of life and frequently leads to the development of osteoarthritis. Tissue engineered strategies have emerged in response to a lack of viable treatments for meniscal pathologies. However, to date, constructs mimicking the structural and functional organisation of native tissue, whilst promoting deposition of new extracellular matrix, remains a bottleneck in meniscal repair. This project aimed overcome these limitations through regional histological and proteomic analysis of native human tissue and transcriptional analysis of their resident cells. 3D bioprinting was then employed to deposit and pattern cellular bioinks with high spatial resolution to enable development of a biomimetic 3D bioprinted meniscal substitute with appropriate structural and functional properties.

Methods
Histology, gene expression and mass spectrometry were performed on native human meniscal tissue to investigate tissue architecture, matrix components, cell populations and protein expression regionally across the meniscus. 3D laser scanning and magnetic resonance imaging were employed to acquire the external geometrical information prior to fabrication of a 3D printed meniscus. Bioink suitability was investigated through regional meniscal cell encapsulation in alginate and blended alginate-collagen hydrogels, with the incorporation of growth factors (TGFβ and/or CTGF) and assessed for their suitability through rheology, scanning electron microscopy, histology and gene expression analysis. Bioprinting of 3D meniscal constructs were fabricated through zonal deposition of regionally tailored bioinks and regional differences in cell and matrix biology assessed.

Results
Meniscal tissue characterisation revealed regional variations in matrix compositions, cellular populations and protein expression. The process of imaging through to 3D printing highlighted the capability of producing a construct that accurately replicated meniscal geometries. Regional meniscal cell encapsulation into bioinks revealed a recovery in cell phenotype, with the incorporation of growth factors stimulating cellular re-differentiation and improved zonal functionality. Bioprinting of bioinks regionally enabled the fabrication of a 3D meniscal construct with regional variations in cell and matrix deposition.

Conclusions
Detailed characterisation of native tissue provides a crucial benchmark for design of novel regenerative therapies. Meniscus biofabrication highlights the potential to print patient specific, customisable meniscal implants. Achieving zonally distinct variations in cell and matrix deposition highlights the ability to fabricate a highly complex tissue engineered construct.

Topic: Biomaterials
Subtopic: Fiber-based biomaterials
Type: Oral presentation

TERMIS25_1235 - Development of a composite scaffold for load-bearing and biomaterial-controlled regeneration of zonal articular cartilage properties

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Introduction/Objectives
Repair of articular cartilage (AC) defects remains a major clinical challenge. Following injury, current treatments cannot effectively repair AC and debilitating degeneration commonly occurs, resulting in pain and loss-of-function. Advanced biomaterials offer a promising alternative, however, engineering biomaterials that provide the mechanical properties required to support load-bearing while promoting cells to adopt AC-like phenotypes remain a challenge. Additive manufacturing techniques like fused deposition modelling (FDM) and melt electrowriting (MEW) enable the engineering of composite biomaterials that integrate load-bearing and regenerative components while preserving their activity. Therefore, this study aimed to combine a layered, but continuous, MEW/FDM reinforcing framework, designed to mimic the zonal mechanical properties of AC, with a corresponding layered, but continuous, hydrogel to enhance the regeneration of functional AC.

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Methods
MEW/FDM scaffolds were fabricated from polycaprolactone and the effects of print design and fiber characteristics on mechanical properties (tensile and compressive) determined. For hydrogels, methacrylated gelatin (GelMA) and hyaluronic acid (MeHA) blends were synthesized and their mechanical and physical properties analyzed. Candidate hydrogels were selected across a range of mechanical properties and human articular chondrocytes (hAC) or mesenchymal stem cells (hMSC) encapsulated. The impact of hydrogel formulation on cytocompatibility, chondrogenic phenotype and extracellular matrix (ECM) composition and deposition was assessed (qRT-PCR, immunoblot, immunofluorescent staining (IFS), histology). The impact of MEW/FDM on cell morphology, alignment and ECM deposition was investigated through IFS and second harmonic generation microscopy.

Results
Mechanically reinforcing MEW/FDM scaffolds exhibited high print fidelity and tunable mechanics, with tensile and compressive moduli relevant for corresponding target AC zones. Designs had significant impact on tensile properties, while fiber spacing had greater influence on compressive properties. Regenerative GelMA/MeHA hydrogels showed significant variation in compressive properties, including Young's moduli (0.8 ±0.2 to 95.3 ±26.3 kPa). Hydrogels across a range of stiffnesses and GelMA:MeHA ratios were selected and their influence on cell differentiation and phenotype assessed. All hydrogel formulations displayed excellent cell viability, with softest formulations increasing metabolic activity compared to stiffest ($P<0.05$; n=4). Hydrogel properties influenced chondrogenic marker expression in culture, with softer formulations promoting aggrecan and collagen II expression and downregulating collagen X. When MEW scaffolds and hAC-laden hydrogels were combined, MEW architecture directed cell and ECM alignment.

Conclusions
MEW/FDM design was able to fine-tune the reinforcing properties of the biomaterial and produce relevant compressive and tensile properties to those exhibited by AC zones, while guiding cell and ECM alignment. Through varying hydrogel formulations, the expression of AC markers could be influenced, providing evidence of improved spatial expression of functional ECM molecules and increased phenotypic control. This approach shows promise for development of zonal biomaterials replicating the cell phenotypes and mechanical properties of healthy AC.

Topic: Tissue-Specific Focus
Subtopic: Cartilage
Type: Oral presentation

TERMIS25_1381 - What are early degenerative changes in articular cartilage und how can they be diagnosed a novel bovine early degeneration model

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Objectives
Due to the limited regenerative capacity of articular cartilage, early diagnosis for tissue-preserving therapy is essential. As degeneration starts before clinical symptoms appear, diagnostic tools are needed to detect early changes before macroscopically visible damage. This study evaluates a multi-photon imaging tool (MPM) to detect early cartilage degeneration without histological processing. Given the high risk of osteoarthritic lesions in the bovine femoropatellar groove (FG), it is hypothesized that early degenerative changes occur in macroscopically healthy bovine knee joints distally in the FG, where higher mechanical pressures occur. Additionally, we hypothesized that current clinical non-invasive imaging tools cannot detect these early changes. Therefore, this study introduces a model of very early degenerative changes in articular cartilage and potential ways to diagnose these in a clinical setting.

Methods
In bovine knee joints (n=8), the FG was divided into six regions (proximal, intermediate, distal; each medial and lateral), from which cartilage-bone cylinders were drilled for superficial layer analysis. SHG and MPAF images were taken in a top-down view, simulating an endomicroscopic and clinically applicable setting. Histological (immuno-)stainings (NITEGE: aggrecan degradation neoepitope; elastin; von Kossa; toluidine blue) of classical sections were performed to validate and quantify (OARSI grading and scoring) degeneration. Calcein staining of the surface analysed cell morphology (CM) and spatial distribution (SCSO). To add clinical non-invasive imaging, X-rays and CT scans (with and without calcium-specific post-processing [SBI]) were performed, along with MRI, including cartilage T2 mapping. Statistical analysis used mixed linear models.

Results
On X-rays and CT-scans and morphologically all six regions revealed no difference to each other. MRI analysis did not reveal different T2 relaxation times. In MPM, however, proximal superficial tissue starts with a cell-free signal-poor layer (level A), followed by fluorescent elastin fibres (level B), and the first layer of cells (level C). In the distal FG a degenerative process was detected by SHG and MPAF. SHG visualized pericellular dot-like signals which tend to be of a protein nature, rather than depositions of calcium, seen in calcium sensitive staining and SBI. Level B was either completely gone or narrowed. Fissures and "cracks" in the collagenous matrix were found. Cell morphology and SCSO varied significantly from proximal tissue and (immuno-)histology confirmed an early degenerative process (NITEGE detection) and slightly increased OARSI grades ($p < 0.01$) in the distal superficial tissue.

Conclusions
In the distal bovine FG, an early degenerative process is visualizable in the superficial articular cartilage, making it a potential model for early degeneration. MPM revealed this in an arthroscopically usable endomicroscopic view. Calcein staining, CM and SCSO also detected differences between proximal and distal regions. The origin and specificity of the pericellular signals remain unclear but appear to be linked to the degeneration. These signals and collagen disruptions are undetectable with current clinical tools like inspection, palpation, or imaging methods, including MRI, despite suggestions that T2 mapping might help identify early osteoarthritis non-invasively. Based on this study endomicroscopic diagnosis might fill this gap.

Topic: Microscopy & Advanced Imaging
Subtopic: Tracking cells, extracellular vesicles
Type: Oral presentation

TERMIS25_1418 - Spatial distribution of extracellular vesicles, autofluorescence, and CD9 positivity around chondrocytes in the superficial layer of articular cartilage

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Objectives
Extracellular vesicles (EVs) are becoming a key research focus in cartilage and osteoarthritis research, yet their precise localization in the tissue remains underexplored. Therefore, this study aimed to investigate the distribution of EVs in superficial healthy cartilage, hypothesizing that EVs a) are not evenly distributed around chondrocytes in this zonally organized tissue and b) share the same pattern as tissue autofluorescence. Therefore, this study helps to understand the topographical relation of EVs and the superficial tissue architecture and forms a basis for the development of tracking methods for EVs in vivo.

Methods
Superficial cartilage tissue from the femoropatellar groove of eight bovine knees was visualized natively using multiphoton microscopy (second harmonic generation, SHG and multiphoton-autofluorescence, MPAF) in the classical zonal section view. Additionally, fixed tissue sections were used for transmission electron-microscopy (TEM) as well as immuno-co-staining for CD9 [membrane marker] and collagen type VI [pericellular matrix (PCM) marker]. Additionally, cartilage-bone cylinders were drilled out of the femoral groove of three knees using a standard drill. They were analyzed using SHG and MPAF in a simulated endo-microscopic setting, generating a view-plane parallel to the tissues surface. Additionally, these cylinders were fixed and cut parallel to the surface reproducing the same view for CD9- and collagen type VI-immuno-stained sections.

Results
In superficial cartilage a repetitive MPAF pattern was found around chondrocytes, with a) accumulations lateral to the cells (which are lying inside SHG-negative lacunae and partly show ascending stripes into the SHG-positive surrounding matrix) and b) "snow-cap"-like formations above the cells outside the lacunae. In TEM EVs were found in a similar form of distribution with vesicle diameters between 50 - 200 nm. These distributional patterns were also visualizable through CD9-staining, additionally revealing that extracellular CD9 was found mainly outside the collagen type VI-positive PCM. The accumulations laterally of the cells were visualizable again in the simulated endomicroscopic setting, and CD9 positive.

Conclusions
EVs and CD9 show a similar, very specific distributional pattern around superficial chondrocytes, with snow-cap-like and lateral accumulations, which suggests a guided transport of EVs through or binding to the ECM. A similar pattern is visualizable via non-labelled multiphoton microscopy suggesting that at least part of the extracellular MPAF is related to EVs. Future studies need to explore the structural cause and role of this specific and correlating distribution of EVs, CD9 and autofluorescence in superficial articular cartilage and whether MPAF might be usable for live-tracking of EVs.

Topic: Tissue-Specific Focus
Subtopic: Cartilage
Type: Oral presentation

TERMIS25_1431 - Advances in nutraceutical strategies for articular cartilage repair: impact of glucosinolates and isothiocyanates on joint cells

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Introduction: Adjuvant therapies based on various natural compounds may offer significant potential for preventing and managing articular cartilage damages (ACDs). Notable bioactive molecules include organosulfur compounds derived from Brassicaceae (OSC), glucosinolates (GLS; precursors) and isothiocyanates (ITC; metabolites). While GLS and their corresponding ITC hold significant potential for cartilage repair, there is currently no clear data comparing their biological activities. This study aims to achieve three key objectives: 1) assess the biocompatibility of GLS versus ITC in diverse cell populations located within the knee joint; 2) evaluate the effectiveness of GLS precursors versus ITC metabolites in driving chondrogenic differentiation; 3) investigate the impact of GLS and ITC on combating inflammatory processes beyond ACDs.
Methods: Human OA chondrocytes, osteoblasts, synoviocytes, and mesenchymal stromal cells (MSCs) were treated with two couples of GLS & ITC (glucoraphanin-GRA & sulforaphane-SFN; benzyl glucosinolate-BGL & benzyl isothiocyanate-BITC). Various concentrations and treatment durations were used to evaluate cell morphology, metabolism, and cytotoxicity. MSCs were also treated in the presence or absence of the selected GLS & ITC during chondrogenesis stimuli to assess their chondroprotective potential (functional analysis, immunohystochemistry). Additionally, we established co-culture systems of chondrocytes and synoviocytes exposed to inflammatory stimuli to investigate their anti-inflammatory activity though real time PCR analysis.
Results: Both GLS and ITC treatments have shown significant benefits for the viability, proliferation, and morphology of all joint cell types at sub-micromolar concentrations. Importantly, at higher concentrations, both GLS and ITC maintained MSC behavior without any detrimental effects. However, it's noteworthy that ITC displayed signs of cytotoxicity when compared to GLS in other cell types, emphasizing the superior safety profile of GLS. Additionally, GLS and ITC-treated MSCs demonstrated remarkable improvements in chondral differentiation. This was evidenced by a substantial increase in the expression of proteoglycans and chondrogenic markers, coupled with a reduction in hypertrophic markers. Notably, GLS compounds outperformed their ITC metabolites in fostering chondrogenic differentiation. Furthermore, both GLS and ITC treatments played a vital role in mitigating inflammatory processes that are known contributors to cartilage degeneration.
Conclusions: This preclinical in vitro study illustrates that GLS and ITC, and especially GLS, can effectively promote chondral differentiation, highlighting their promising role as adjuvant therapies for ACDs. Furthermore, we underscore the crucial need for research on nutraceutical compounds to consider the diverse joint cells collectively. By doing so, we can pinpoint therapeutic concentrations that benefit specific cell types while avoiding cytotoxic effects on others, paving the way for more targeted and effective treatments.

Topic: Submit to SYMPOSIUM
Subtopic: Cell-Based or Cell-free Regenerative Approaches to counteract Intervertebral Disc Degeneration
Type: Oral presentation

TERMIS25_250 - Extracellular vesicles and complement inhibition alter the gene expression profile of intervertebral disc cells in an ex vivo degenerative spine model

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Introduction/Objectives
The terminal complement complex (TCC), a key component of innate immunity, promotes cell lysis and inflammation, and is involved in intervertebral disc (IVD) degeneration (IVDD) (Teixeira et al., 2021). This study explored the role of TCC in IVDD and its modulation. Previous studies have shown that the secretome from interleukin (IL)-1β-primed mesenchymal stem cells (MSCs) can regulate complement activity, inflammation, and matrix metabolism in IVD cells (Neidlinger-Wilke et al., 2021). Taking this into consideration, the therapeutic potential of extracellular vesicles (EVs) derived from IL-1β-primed MSCs was investigated in spine cultures from mice deficient in C6 (Bhole and Stahl, 2004), a TCC component.

Methods
MSCs from C57BL/6J mice were cultured with or without 1 ng/mL IL-1β for 48h (n=3), and EVs were isolated from their secretome by

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ultracentrifugation. Control- and IL-1β-EV were characterized for morphology and size. Secretome and EVs were analysed by proteomics. Mouse lumbar spines from both C57BL/6J wild-type and C6-deficient mice (n=4-6) were then cultured with either i) mouse serum alone (MS), or including ii) IL-1β, or iii) IL-1β+IL-1β-EV. After two days, gene expression of anti-apoptotic (*Bcl2*), complement regulatory (*Cd46*, *Cd55*, *Cd59*), inflammation (*Il6*), and matrix metabolism (*Mmp3*, *Col1a1*, *Col2a1*) markers was measured. IVDD was assessed after 14 days through histological staining and a modified Thompson scale. Statistical analysis was conducted using the Kruskal-Wallis or one-way ANOVA test (significance, $p<0.05$).

Results
Control- and IL-1β-EV displayed typical size distribution (diameter: 105±53 nm, polydispersity index: 0.30). Proteomic analysis indicated distinct protein profiles in IL-1β-Sec and IL-1β-EV, with 36 proteins shared between IL-1β-EV and IL-1β-Sec. IL-1β-EV were enriched in pathways related to inflammatory response regulation, complement system processes, and extracellular matrix organization. In IVDs from wild-type mice, IL-1β stimulation upregulated pro-inflammatory and catabolic markers *Il6* and *Mmp3* ($p<0.05$). In contrast, IL-1β-EV treatment upregulated the anti-apoptotic gene *Bcl2* and the complement regulator *Cd55* compared to the control and IL-1β groups ($p<0.05$). IL-1β increased degeneration scores in the annulus fibrosus and the nucleus pulposus ($p<0.05$), but these scores remained similar to control levels with IL-1β-EV treatment. In IVDs from C6-deficient mice, IL-1β upregulated *Il6* and *Mmp3* ($p<0.05$), while IL-1β-EV treatment mitigated these effects, reducing the inflammatory response without significantly altering degeneration scores.

Conclusions
This organ culture model enabled the investigation of IVDD ex vivo while incorporating TCC deficiency to examine complement-mediated inflammation's role in tissue degradation and repair. IL-1β priming notably altered the molecular composition of MSC-derived EVs, with enrichment in processes and pathways associated with complement regulation. In C6-deficient spine cultures, IL-1β-EVs reducel local inflammation and tissue degeneration, suggesting that TCC may counteract the beneficial effects of EVs. These findings indicate the therapeutic potential of IL-1β-EVs as a novel treatment for spinal disorders associated with complement dysregulation and validate the mouse spine culture as a promising research model prior to in vivo studies.

Topic: Tissue-Specific Focus
Subtopic: Intervertebral disc & spine
Type: Oral presentation

TERMIS25_440 - Characterisation of the human intervertebral disc microenvironment and recapitulated in vitro cellular response

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Introduction/Objectives
Recent therapies for intervertebral disc (IVD) degeneration aimed at restoring matrix and disc height[1] often neglect the intradiscal microenvironment which is vital for cell viability and function[2,3]. The objective of this work is to profile this microenvironment across mild and moderate degenerative states, quantifying glucose, oxygen, pH, lactate, osmolarity, and 13 cytokines, of both pro-regenerative and inflammatory nature, to facilitate therapy development in a more physiologically relevant maner in vitro. Subsequently, cellular synthesis and metabolic rates in response to the recapitulated microenvironments will be analysed to refine predictive modelling and enhance accuracy in forecasting regenerative therapy outcomes in the IVD.

Methods
Human nucleus pulposus (NP) tissue was collected through informed consent of patients undergoing discectomy procedures and approved by the Mater Misericordiae Hospital IRB (Ref 1/378/2229) and Trinity College Dublin (TCDFSTEMSREC/15032021/Buckley). pH was measured in tissue samples using the PreSens pH-1 micro and accompanying needle-type pH microsensor NTH-HP5 (Presens, Germany), oxygen was quantified through fluorescent based hypoxia labelling using EF5 (Sigma-Aldrich). A biochemical assay for quantifying glucose was developed using Glucose Liquid (Sentinel Diagnostics 17630H), and comparatively Lactate was quantified using Lactate Dry Fast (Sentinel Diagnostics 17285). Osmolarity was measured using a vapor pressure osmometer (Vapro ELITechGroup). TNF-α, IL-1β, IL-6, MMP-3, βNGF, BNDF, IL-10, TIMP-1, TIMP-2, and FGF were quantified using a custom multiplex assay (Assay Genie) read using flow cytometry. ADAMTS4, ADAMTS5, and TIMP-3 were quantified using ELISAs (Assay Genie).

Microtissues of 50,000 cells were formed using primary cells isolated from the tissue samples. Following 3 days of formation, the microtissues were primed for 7 days with TGF-β3. Cellular insult was conducted at 3 glucose levels and 2 pH levels corresponding to the previous measurements for 14 days post priming. Microtissues were then assessed for viability, DNA, GAG, and collagen content.

Results
pH was the only measured factor that significantly changed as a function of degeneration with mild degeneration measuring 7.52 ± 0.14 and moderate degeneration at 6.92 ± 0.62. Logistical regression indicated a trend in TNF-α, IL-6, ADAMTS5 and TIMP-3. Glucose and pH did not have a significant impact on cellular synthesis rates or viability within a physiologically relevant range.

Conclusions
Microenvironmental factors within the human IVD were quantified across mild and moderate degeneration, with no significant differences noted. Oxygen, pH, osmolarity, and lactate levels were found to be consistent with previous studies [4, 5]. To the best of the authors knowledge, this study marks the first experimental quantification of glucose in human tissue, with results aligning with predicted ranges from in silico models [6]. The cellular response was not adversely affected by microenvironmental glucose and pH concentrations suggesting broad applicability for regenerative therapies.

References:
[1]Vedicherla, et al., (2017), [2]Schol, et al., (2019), [3]Sakai, et al., (2017), [4]Bartels et al., (1998), [5]Sadowska et al., (2018), [6] McDonnell et al., (2022).

Acknowledgements:
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Topic: Submit to SYMPOSIUM
Subtopic: Cell-Based or Cell-free Regenerative Approaches to counteract Intervertebral Disc Degeneration
Type: Oral presentation

TERMIS25_782 - Differentiation of human bone marrow-derived mesenchymal stromal cells towards nucleus pulposus-like cells: The role of 3D culture

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Introduction/Objectives Low back pain (LBP) is a significant global health issue, primarily caused by intervertebral disc degeneration (IVDD), which is influenced by trauma, diseases, and aging. As the population ages, the prevalence of IVDD is expected to increase, impacting the quality of life and imposing economic burdens on communities. Stem cell-based strategies for tissue regeneration include direct cell injection and tissue engineering, with the latter offering improved cell retention and differentiation. Hydrogels have been used to enhance stem cell engraftment. Microsized hydrogels, called microgels, have been developed to provide advantages such as high surface-to-volume ratio and injectability. Hyaluronic acid (HA), recognized for its biocompatibility and biodegradability, has been utilized in clinical applications for over three decades. This study explores the effect of a 3D microenvironment on bone marrow-derived mesenchymal stromal cells (BM-MSCs) differentiating into Nucleus Pulposus (NP)-like cells to promote IVD regeneration. We encapsulated BM-MSCs in HA-based bulk hydrogel and microgel, assessing the impact of NP differentiation factors GDF-5 and TGFβ1.

Methods Hyaluronic acid was modified with tyramine groups (HA-Tyr) [2] and mixed with horseradish peroxidase (HRP) and BM-MSCs. Microdroplets were generated using a flicking-based vibrating nozzle system and collected in a hydrogen peroxide (H2O2) solution to form microgels. A hydrogel solution was also dropped in a crosslinking bath to produce macrogels. These 3D cell-encapsulated carriers were cultured for 21 days in a differentiation medium containing 2% fetal bovine serum, GDF6, and TGFβ1, with control groups consisting of cell pellets without hydrogel.

Results Cell viability was high in pure HA-microgels and macrogels; however, BM-MSCs did not proliferate. In both groups, the microgel and macrogel groups, the cells displayed a rounded morphology resembling that of Nucleus Pulposus (NP) cells. Phenotypic analysis indicated that all groups enhanced the expression of NP markers, including collagen II, Sox 9, and aggrecan. While the expression of these markers was significantly higher in the microgel and hydrogel groups compared to their respective controls. There was no notable difference between the microgel and macrogels. Furthermore, the differentiation medium, enriched with GDF6 and TGFβ1, significantly boosted the expression of NP markers compared to the basal medium, with earlier and higher levels of expression observed.

Conclusions Our findings demonstrate that combining the two growth factors (GDF5 and TGFβ1) and encapsulation within HA-based hydrogel/microgel synergistically enhances the differentiation of BM-MSCs into NP cells. The absence of a significant difference in outcomes between the microgel and macrogels may be due to the high porosity of crosslinked HA-Tyr, which facilitates nutrient and metabolite diffusion in both groups. This study underscores the potential of using HA-based hydrogels/microgels for effective tissue engineering therapies in regenerative medicine.

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Topic: Submit to SYMPOSIUM
Subtopic: Cell-Based or Cell-free Regenerative Approaches to counteract Intervertebral Disc Degeneration
Type: Oral presentation

TERMIS25_1085 - Comparative Transcriptomics of Tie2-Positive Progenitor Cells in the Nucleus Pulposus

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Introduction/Objectives
Intervertebral disc (IVD) degeneration (IDD) is a major cause of back pain and disability, with limited treatment options due to a limited understanding of IVD biology. TEK, the gene encoding the Angiopoietin-1 receptor (Tie2), identifies a progenitor cell subset within the nucleus pulposus (NP) crucial for repair and regeneration. This study investigates the transcriptomic profiles of Tie2-positive (Tie2pos) respectively Tie2-enriched (Tie2enr) NP cells (NPCs), which are thought to be progenitor cells compared to other NPC poulations, to elucidate their roles in IVD homeostasis and regeneration.

Methods
Human and bovine Tie2pos NPCs were isolated and enriched via enzymatic digestion followed by fluorescence-activated cell sorting (FACS). Bulk RNA sequencing (RNAseq) was conducted on human Tie2enr, Tie2-negative (Tie2neg), and unsorted NPCs following a seven-day expansion period. For bovine NPCs, cells were pooled from six animals to achieve sufficient cell numbers, followed by FACS and bulk RNAseq without expansion. Differential gene expression (DGE) analysis was performed using DESeq2, and pathway enrichment analysis identified associated biological processes and pathways. Single-cell RNAseq data from bovine NPCs, previously published, were reanalyzed using Seurat, revealing 14 transcriptionally distinct cell clusters. These data were integrated with the bulk transcriptomics to refine findings.

Results
Pathway enrichment analyses highlighted key biological processes and pathways associated with Tie2pos and Tie2enr cells. These included cellular interactions with the extracellular matrix (ECM), vascular processes, and signaling pathways relevant to tissue repair and progenitor activation. Species-specific differences and the impact of monolayer culture were observed, underscoring the complexity of Tie2pos cell biology. Specific transcriptional signatures associated with inflammation, ECM remodeling, and growth factor signaling were identified.

Conclusions
These findings provide insights into the tanscriptomic landscape of bovine Tie2pos and human Tie2enr NPCs, emphasizing their supposed roles in ECM integrity, progenitor activity, and tissue repair within the avascular IVD environment. This work contributes to the understanding of Tie2pos NPCs and their potential applications in IVD regeneration.

This work was supported by the Swiss National Science Bridge Discovery Project # 40B2-0_211510/1 (https://data.snf.ch/grants/grant/211510).

Topic: Submit to SYMPOSIUM
Subtopic: Cell-Based or Cell-free Regenerative Approaches to counteract Intervertebral Disc Degeneration
Type: Oral presentation

TERMIS25_1262 - Exploring CD44+ intervertebral disc cells under homeostatic and degenerative settings as new therapeutic targets for discogenic pain

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Introduction/Objectives
Low back pain (LBP) is a leading cause of disability worldwide[1]. Discogenic pain is a form of LBP without spine deformation and

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associated with strong inflammation[2]. Its unclear underlying mechanisms lead to the lack of specific therapeutic targets and low efficacy of current treatments. Discogenic pain is correlated with degeneration of the intervertebral disc (IVD), comprised by a variety of heterogeneous cellular populations, including a rare myeloid-derived subset, although their contribution to discogenic pain is not clearly defined. Previous work from the group has shown that CD44, a cell surface glycoprotein involved in cell-extracellular matrix (ECM) interactions[3], is expressed by nucleus pulposus (NP) cells and increased upon inflammatory stimuli[4].

Given the significance that inflammation has in discogenic pain, the aim of this work was to further characterize CD44+ IVD cells, exploring their spatial distribution and association with myeloid cell markers, relevant in the IVD inflammatory cascade.

Methods
Bovine IVDs were collected, with NP and annulus fibrosus (AF) separated and digested for cells isolation[5]. NP and AF cells were maintained in 2D culture under both homeostatic and interleukin-1β (IL-1β)-mediated (10 ng/ml) pro-inflammatory conditions. Additionally, NP and AF explants were maintained in culture, inducing degeneration by 21G needle puncture and IL-1β (10 ng/mL)[5]. Cell phenotype was characterized by flow cytometry and immunohistochemistry (IHC) for a panel of markers: CD44, CD45 (pan-hematopoietic cells), CD14 (monocytes/macrophages lineage), MHC-II (cell activation marker), CD24 (notochordal cells) and GD2 (IVD progenitor cells).

Results
In fresh IVD cells, CD44 was more expressed in AF (20±5%) than NP (9±6%), as well as MHC-II (36±21% in AF and 28±24% in NP), with low frequencies of CD14, CD24 and GD2 (<5%) and no expression of CD45. These markers' expression and their spatial location are currently being confirmed by IHC. Under 2D culture, NP and AF cells maintained both adherent and suspension viable cells, although the expression of all surface markers decreased overtime in both populations. Moreover, a subpopulation of CD44+CD14-MHC-II+CD45- NP suspension cells increased with inflammation in 2D culture (to 23±12%). Interestingly, CD44 expression revealed to be higher in IVD organ culture (33%±14 in NP and 25±5% in AF), with the influence of hypoxia on CD44 expression also being investigated. Finally, CD44+ NP cells, but not CD44+ AF cells, increased under degenerative/pro-inflammatory conditions in organ culture (to 38±17%).

Conclusions
This work emphasizes that CD44+ IVD cells can be an important immunomodulatory target in IVD degeneration, strongly associated with the presence of IVD ECM. It also suggests that healthy IVD does not present resident myeloid-derived cells. Further studies are being conducted to address the targeting of CD44+ IVD cells.

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Topic: Submit to SYMPOSIUM
Subtopic: Cell-Based or Cell-free Regenerative Approaches to counteract Intervertebral Disc Degeneration
Type: Oral presentation

TERMIS25_1467 - Isolation and Characterization of Extracellular Vesicles from bovine Tie2+ and Tie2- Nucleus Pulposus Cells

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Introduction/Objectives
Intervertebral disk (IVD) degeneration (IDD) is a significant contributor to low back pain (LBP), the leading cause of disability worldwide. IDD is a complex and multifactorial process, rendering the selection of the appropriate therapy difficult. While current therapies focus on conservative or surgical methods, their effectiveness remains debatable. Novel regenerative approaches focus on a recently discovered nucleus pulposus (NP) progenitor cell (NPPC) population, positive for Tie2. Tie2+ cells show regenerative and properties. Together with these progenitor cells, extracellular vesicles (EVs) have also gained considerable interest lately as a new way to treat LBP. EVs are particles, released from cells, delimited by a lipid bilayer and incapable of replication. They are known to carry various cellular and thus play a pivotal role in cellular communication. It has been shown recently that human nucleus pulposus cells (NPCs) derived EVs exhibit different features than EVs secreted by human mesenchymal stromal cells (MSCs) and are more efficient in enhancing cell proliferation in degenerated IVDs. Thus, this study aims to compare and characterize the EVs secreted from three different bovine cell sources: NPCs (Tie2+ and Tie2-) and donor matched adipose tissue derived MSCs (AdMSCs).

Methods
NPCs and AdMSCs were isolated from bovine tails (n=4) obtained from a local abattoir by isolating the NP and the adipose tissue. While MSCs were seeded directly, NPCs were sorted using fluorescence-activated cell sorting (FACS) to obtain Tie2+ and Tie2- populations. Tie2+ and Tie2- NPCs were functionally compared by colony forming unit (CFU) assays and by bi-lineage differentiation into adipocytes and osteoblasts. AdMSCs were tri-lineage differentiated additionally into nucleopulpocytes. All cells were passaged once and cultured until 80% confluency. The standard medium was switched to EV-free medium for 2 days before harvesting for downstream proteomics and transcriptomics. EVs were isolated from the conditioned media using ultra-centrifugation followed by size exclusion chromatography, and protein concentration was determined by Smith assay.

Results
FACS revealed a range of 0.1% to 12.3% Tie2+ cells within the NPC population. EVs were successfully isolated from bovine-derived NPCs (Tie2+ and Tie2-) and AdMSCs, with average yields of $1x10^9$, $0.8x10^9$ and $1.43x10^9$ particles in 0.5 mL, respectively. Nanoparticle tracking analysis revealed consistent purification of EV populations averaging 191, 193 and 176 nm in size, with a mean zetapotential of -29.2, -27.6, -29.2 mV, respectively. Current analyses on bulk RNAseq and proteomics, as well as CFU and differentiation assay, are ongoing.

Conclusions
Our preliminary results suggested EVs could be reliably isolated from different bovine cell sources. Healthy bovine cell and tissue sources allow us to investigate the regenerative effect of EVs in an *ex vivo* IVD organ culture model within the same species. Bovine-derived Tie2+ derived EVs will be a pre-clinical model to test their regenerative potential compared to other cell sources, e.g., AdMSCs.

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Topic: Submit to SYMPOSIUM
Subtopic: Frontiers in Musculoskeletal Regeneration
Type: Oral presentation

TERMIS25_576 - Secretome of bone marrow- and umbilical cord-derived mesenchymal stromal cells cultured in different culture systems and its effect on cartilage regeneration

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Introduction/Objectives
Damage to articular cartilage (AC) caused by accidents, sports injuries and age-related wear and tear often leads to osteoarthritis (OA). Due to a limited natural regenerative capacity resulting from lacking vascularisation, low chondrocyte density and released pro-inflammatory messengers, initial microtraumas and lesions fail to heal adequately and deteriorate continuously. Established therapies have shown low efficacy in restoring AC extracellular matrix (ECM). In addition, they often require two-stage surgery linked to infection risks, repeated anaesthesia and increased costs. Thus, we tested a novel therapy approach using the secretome of mesenchymal stromal cells (MSCs) to stimulate *in vitro* AC ECM production by chondrocytes. As the presence of secretome-containing trophic and immunomodulatory factors depends on MSC source and extracellular conditions, human bone marrow-derived MSCs (hBM-MSCs) and human Wharton's Jelly-derived MSCs (hWJ-MSCs) were cultured as monolayers and in four different 3D *in vitro* culture systems representing carriers for MSC insertion into and retention in damaged AC. We investigated whether a secretome supporting cartilage regeneration could be observed.

Methods
hWJ-MSCs and hBM-MSCs were cultured for 21 days in different culture systems including spheroids, alginate beads, porous collagen I scaffolds and commercially available bilayer collagen membranes (Chondro-Gide®, Geistlich Biomaterials) as well as in monolayers. Collected secretome-enriched conditioned medium (CM) was transferred every 3 to 4 days to pellets of chondrocytes isolated from cartilage fragments of the human knee joint. Chondrocyte pellets cultured with fresh chondrogenic medium served as controls. Analyses included LIVE/DEAD staining of 3D-cultured MSCs, ELISA for interleukin 10 and 13 and growth differentiation factor 5 (GDF-5) in CM, as well as gene expression and protein secretion analyses for chondrogenic and ossification markers in chondrocytes.

Results
MSCs showed adequate viability, although elevated presence of dead cells was evident after 21 days. In the CM, only GDF-5 was detectable with increased secretion by hWJ-MSCs compared to hBM-MSCs. We did not observe a significant increase in gene expression of chondrogenic markers aggrecan, cartilage oligomeric matrix protein, collagen II and SRY-box transcription factor 9 in chondrocytes cultured with CM of 3D-cultured MSCs but partly in chondrocytes with CM of MSC-monolayers. Gene expression of ossification markers often showed a significantly higher expression of alkaline phosphatase compared to the control, which was not evident for collagen I and X. Qualitative analysis of protein secretion by histological staining and quantitative analysis by collagen II-ELISA and sulphated glycosaminoglycan-assay did not show increased AC ECM production.

Conclusions
Secretome obtained from differently cultured MSCs did not significantly support cartilage regeneration. Results might be due to a senescence-associated phenotype, an inadequate cell/medium ratio or the interaction/binding of secreted factors with/to biomaterials. Transferring the method to co-cultures might result in different effects due to intercellular communication. Further, biomaterial modifications may provide a basis for improving extracellular conditions, potentially leading to a desirable secretome driving the establishment of secretome-based therapy for AC damage.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_734 - Multifunctional Injectable Hydrogel Accelerates Fracture Repair Through Sustained Release of PTH (1-34) Pre-conditioned Umbilical Cord Mesenchymal Stromal Cell-derived Extracellular Vesicles

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Introduction/Objectives
Bone nonunion remains a significant clinical challenge requiring urgent solutions. Recent studies have highlighted the critical role of the metabolic microenvironment in fracture healing. Stem cell-derived extracellular vesicles (EVs), naturally occurring nanoparticles containing bioactive molecules, have emerged as promising cell-free therapeutic agents for promoting bone regeneration. EVs derived from human umbilical cord mesenchymal stem cells (hUCMSCs) have shown potent activity in enhancing bone marrow stromal cell (BMSC) recruitment and proliferation. However, issues such as efficient delivery, sustained release, and the preservation of EVs activity have limited their clinical application.

Methods
EVs derived from hUCMSCs and preconditioned with parathyroid hormone (PTH pre-EVs) were collected. Dopamine was self-polymerized on the surface of both EVs, improving drug-loading capacity and pharmacokinetics. The polydopamine-coated EVs (PDA-EVs) were then integrated into a hyaluronic acid (HA) and sodium alginate (SA)-based hydrogel, an injectable, self-healing, adhesive, and multifunctional HA/SA/PDA-EVs hydrogel obtained.

Results
The hydrogel demonstrated excellent biocompatibility, hemostatic properties, and antibacterial activity. The controlled and sustained release of EVs, particularly PTH pre-EVs was achieved from the hydrogel, which significantly promoted endochondral ossification and macrophage reprogramming both in vitro and in vivo. Mechanistically, PTH-EVs enriched calcifediol enhanced transcriptional activities of SOX9 and BMP2 in BMSCs while reprogramming macrophage oxidative phosphorylation to accelerate tissue repair.

Conclusions
In conclusion, we successfully developed a multifunctional, osteoinductive EVs-loaded hydrogel that orchestrates the bone healing microenvironment to promote bone regeneration. This novel approach provides a promising strategy for tissue engineering and the clinical treatment of bone fractures.

Topic: Biomaterials
Subtopic: Hydrogels
Type: Oral presentation

TERMIS25_982 - Advancing human placenta-derived ECM hydrogels for tissue engineering and regeneration

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Objectives
Efforts to regenerate or create soft tissues requires bioactive scaffolds with mechanical stability and cell-supportive properties. This study addresses the challenges associated with extracellular matrix (ECM) hydrogels, focusing on the development and improvement of human placenta ECM (hpECM) hydrogels. While ECM hydrogels are generally composed of bioactive proteins that facilitate cell-matrix interaction, concerns remain regarding biomechanical strength, reproducibility and ethical considerations if animal tissues are used. Therefore, this study investigates the potential of hpECM as an ethically acceptable and valuable human tissue biomaterial for clinically relevant applications in tissue engineering and regeneration.

Methods
The production of hpECM hydrogels requires a precise process that includes tissue removal, decellularization, enzymatic digestion and sterilization. Our hpECM hydrogels have been tested for protein composition, cytocompatibility and functionality in a wide range of in-vitro and in-vivo applications. Hydrogel blends combining hpECM with silk fibroin or fibrinogen have been evaluated for improved biomechanical properties while maintaining their high bioactivity. Studies with human primary cells encapsulated in the hpECM hydrogels and 3D bioprinting experiments were performed to evaluate their feasibility to produce tissue scaffolds.

Results
We successfully established a reproducible protocol for producing hpECM hydrogels from various regions of the human placenta, resulting in four distinct hydrogels with unique protein compositions. These hpECM hydrogels demonstrate high consistency in both composition and quality. Proteomic analysis confirmed the retention of key matrisome proteins specific to the source tissue, alongside effective decellularization, ensuring minimal DNA content. In various concentrations, hpECM hydrogels are suitable for surface coating, as injectable hydrogels, for 3D cell culture or as bioinks for 3D bioprinting. Initial 3D cell culture experiments showed uniform cell distribution and high cytocompatibility in hpECM hydrogels, underlining their potential as bioinks for 3D bioprinting. The biomechanical properties of hpECM blended hydrogels containing silk fibroin or fibrinogen showed promising improvements in biomechanics of the material. State-of-the-art 3D bioprinting techniques in combination with our hpECM bioinks made it possible to produce complex structures, including microchannels, porous scaffolds and physiological-sized heart tissue models. In-vivo application showed high biocompatibility of our material.

Conclusions
Our hpECM bioinks hold great promise for developing advanced in vitro models that closely mimic in vivo processes and improve current test systems. As a human-derived material, placental ECM offers unique clinical relevance and provides an abundant, ethically sourced resource for tissue engineering and regenerative medicine.

Topic: Submit to SYMPOSIUM
Subtopic: Cells, secretomes, and extracellular vesicles: moving perinatal biomaterials towards clinical translation
Type: Oral presentation

TERMIS25_1407 - Pro-regenerative miRNAs from human amniotic membrane as a potential source for tissue regeneration

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Introduction/Objectives
The human amniotic membrane (hAM) has been recognized as a promising source of therapeutic cells in tissue regeneration for over a century. The cells have been attributed with stem cell characteristics. However, procedures such as cell isolation and *in vitro* expansion may lead to genomic instability and loss of intrinsic properties. A promising alternative to the isolated cells, is the usage of cell-derived bioactive factors released directly from the viable native hAM. Regarding hAM derived miRNAs, one major group of molecules that are crucial in cell communication in health and disease, only little information is available. Therefore, the aim of this project is to characterize pro-regenerative miRNAs in the hAM secretome.

Methods
In order to study EV-contained and protein-bound miRNA, supernatants from hAM biopsy punches of placental amnion (P) and reflected amnion (RA) were collected after 72 h and subjected to size exclusion chromatography (SEC). EV fractions were characterized for particle concentration and size distribution by nanoparticle tracking analysis (NTA) and fluorescence-triggered flow cytometry (FT-FC) for EV content, size, and composition. Surface markers were confirmed with Transmission Electron Microscopy (TEM) and Western blot (WB). On the other hand, protein fractions were characterized for particle concentration and size distribution by NTA, and the protein concentration was measured by microBCA assay. Tissue and supernatants were analyzed by RNA sequencing.

Results
For both P and RA, FT-FC and NTA confirmed the presence of particles smaller than 200 nm (75 % of EVs). CD81 was prominent in all samples (24 %, n = 2, biological replicates). WB and TEM confirmed the presence of CD81 (n = 3, biological replicates). Our results showed three main clusters of miRNAs differentiating tissue miRNAs, EV-contained miRNA and protein-bound miRNA. Subclusters of miRNA correlating with the hAM subregions (P and RA), both in the secretome and the tissues. Differentially expressed miRNA between hAM subregions and between tissue, EV-contained and protein-bound miRNA were also identified. Pathway enrichment analysis of these differentially expressed miRNA revealed statistically significant pathways related to skin and bone regeneration, including osteoblast regeneration, wound healing, and immunomodulation among others.

Conclusions
In our next step, we want to further investigate the secretome related to the regenerative potential of hAM using *in vitro* models pursuing a potential therapeutic approach to support tissue regeneration.

Topic: Biomaterials
Subtopic: Ceramics
Type: Oral presentation

TERMIS25_71 - Effects of strontium and cerium substitution on the properties of bioactive glass-containing scaffolds for bone tissue engineering

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Introduction/Objectives
Bioactive glasses (BGs) have been extensively utilized in treating bone defects due to their ability to bond and integrate with both hard and soft tissues. The combined effects of Si, Ca, and P ions released from BGs enhance the differentiation of osteoblasts by activating signaling pathways associated with osteogenesis. BGs are doped with therapeutic inorganic ions to improve their properties, with cerium (Ce) and strontium (Sr) being particularly noteworthy due to their unique material and biological characteristics. Ce- and Sr-doped BGs are currently being investigated as potential therapies for bone tissue treatment, demonstrating possible benefits such as antioxidant, antibacterial, osteogenic, and angiogenic properties.

Methods
This research aimed to investigate the impact of incorporating Ce/Sr into BG on the physical, chemical, and biological properties of gelatin-based scaffolds, in comparison to samples containing only BG45S5. The biocompatibility of the scaffolds was evaluated using human bone marrow-derived mesenchymal stem cells (hBM-MSCs) through MTT analysis. The osteogenic differentiation of hBM-MSCs cultured on the scaffolds was assessed by measuring alkaline phosphatase (ALP) activity and the expression of genes associated with bone formation. The osteogenic

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characteristics of the Sr-containing scaffolds were evaluated in rabbit calvarial bone defects over 12 weeks. Additionally, the growth inhibition against Escherichia coli (*E. coli*) and Staphylococcus aureus (*S. aureus*) cultured on Sr-doped BG scaffolds was investigated.

Results
The mechanical properties and bioactivity of the scaffolds were enhanced with the addition of BG/Ce. The results also confirmed the biocompatibility and osteoinductive potential of BG/Ce-containing scaffolds for applications in bone tissue engineering. Antibacterial investigations demonstrated that the incorporation of Sr significantly improved antibacterial activity against both S. aureus and E. coli. Implantation of the scaffolds into rabbit cranial bone defects for 12 weeks resulted in increased bone formation, as confirmed by histological analysis.

Conclusions
Our results confirmed that Sr/Ce-doped bioactive glasses (BGs) are promising candidates for bone tissue engineering due to their superior mechanical and antibacterial properties, as well as their enhanced capacity for bone regeneration compared to similar BGs without added ions.

Topic: Submit to SYMPOSIUM
Subtopic: Biomimetic and Bioinspired Materials for Regenerative Medicine
Type: Oral presentation

TERMIS25_507 - Comparative study of ectopic osteogenesis induced by rhBMP6 in autologous blood coagulum with synthetic ceramics in rats and rabbits

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Introduction/Objectives
Bone morphogenetic proteins (BMPs) are highly osteoinductive molecules frequently used in clinical settings to promote bone regeneration. Recently, we developed Osteogrow-C, an innovative autologous bone graft substitute that combines recombinant human Bone Morphogenetic Protein 6 (rhBMP6) within an autologous blood coagulum (ABC), serving as a BMP carrier, with synthetic ceramics acting as a compression-resistant matrix. This study aims to assess the time-dependent progression of ectopic osteogenesis in a rat subcutaneous assay and a rabbit posterolateral spinal fusion (PLF) model induced by Osteogrow-C implants.

Methods
Osteogrow-C implants were prepared as follows: lyophilized rhBMP6 (20 μg per implant in rats and 125 μg in rabbits) was dissolved in water and mixed with freshly collected autologous blood. The blood containing rhBMP6 was combined with biphasic calcium phosphate ceramic particles (500-1700 μm in size) and allowed to coagulate. Osteogrow-C implants were placed subcutaneously in the axillary regions of Sprague Dawley rats and between the transverse processes of New Zealand White rabbits. Rats were euthanized at 7, 14, 21, 35, and 50 days post-surgery, while rabbits were euthanized at 7, 14, and 27 weeks post-surgery to examine the sequence of ectopic osteogenesis. Extracted specimens underwent microCT scanning and histological processing.

Results
In the rat subcutaneous assay, microCT analyses showed extensive bone formation 14 days after Osteogrow-C implantation. Histological examination revealed active endochondral ossification at the implant periphery 7 days post-surgery, while by day 14, newly formed bone extended throughout the implant and around ceramic particles. By day 21, ectopic osteogenesis induced by rhBMP6 reached its final stage, with cortical bone formation and bone present on ceramic surfaces, within pores, and between particles. At day 50, the structural properties of newly formed bone were similar to those at day 21, although the number of adipocytes increased, becoming the predominant cell population in the bone marrow. In the rabbit PLF model, the spinal fusion success rate was over 90% at 7 weeks and reached 100% at 14 and 27 weeks post-implantation. At 7 weeks, newly formed bone was present between ceramic particles, while the cortices were discrete. By 14 and 27 weeks, the cortical bone thickened, while the amount of bone between particles decreased, accompanied by bone marrow adipogenesis.

Conclusions
This study elucidates the dynamics of ectopic osteogenesis triggered by rhBMP6 in an ABC with ceramics (Osteogrow-C). Osteogrow-C implants containing ceramic particles (500-1700 μm) promoted bone formation at the ectopic site in rats and achieved spinal fusion in rabbits, demonstrating excellent osteoinductive properties. The sequence of events in ectopic osteogenesis was similar in rats and rabbits, though the process was significantly slower in the larger animal model.

Topic: Biomaterials
Subtopic: Ceramics
Type: Oral presentation

TERMIS25_670 - Degradation processes of carbonate and silicate-doped hydroxyapatites: an in vitro and in vivo study

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Introduction/Objectives
Calcium phosphate (CaP)-based bioceramics, such as hydroxyapatite (HA) or β-tricalcium phosphate (β-TCP), are widely and successfully used in cranio-maxillofacial and orthopedic surgery because they have a chemical composition similar to the mineral phase of bone, making them biocompatible, osteoconductive and potentially osteoinductive. Doping HA with bioactive ions is a promising strategy to modulate the properties of the HA-based bioceramics and, thus improve their performance. More specifically, carbonate ions, the most abundant ion substitute in bone mineral, promote HA resorption when incorporated into the ceramic lattice. The silicate ion is also of interest because its bioavailable form, $Si(OH)_4$ has been shown to stimulate osteogenic differentiation and bone mineralization. Silicate-doped hydroxyapatites (SiHAs) have been extensively studied but remain as poorly soluble as HA. Co-doping HA with both silicate and carbonate ions could combine the advantages of both by allowing optimal degradation of the material while enhancing bone regeneration. This combined doping strategy remains largely unexplored and the degradation potential of monophasic carbonate and silicate-doped HA (CSiHA) has never been evaluated. Therefore, the aim of this study was to evaluate the degradability of CSiHA bioceramics and to identify the primary mechanisms involved, such as dissolution or osteoclast-mediated resorption.

Methods & Results
Five ceramic compositions (HA, $Si_{0.4}$HA, $C_{0.8}$HA, $C_{0.8}Si_{0.4}$HA, and β-TCP) were produced as 200-to-500μm granules and implanted subcutaneously in mice for 6 months to assess degradation in a non-osteogenic environment. Both μCT monitoring and post-mortem weighing of the remaining ceramic demonstrated not degradation but rather a mass increase: 40% for HA and SiHA, 20% for CHA and CSiHA, and 0% for β-TCP. This mass gain, indicating a surface precipitation mechanism[7], was confirmed by SEM analysis, which also showed an intergranular and intragranular dissolution on β-TCP ceramic and only an intergranular dissolution (i.e., at grain boundaries) on CHA and CSiHA ceramics. To evaluate the ceramic degradation in an osteogenic environment, the granules were implanted subcutaneously with osteoprogenitor cells that induce bone formation and recruit osteoclasts. Eight weeks post-implantation, the resorption rates (v/v) quantified by μCT were negligible for HA and SiHA, 10% for CHA and CSiHA, and 30% for β-TCP. TRAP

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staining identified osteoclasts on the granule surfaces, suggesting a cell-mediated degradation.

Besides, *in vitro* studies were conducted to investigate the influence of HA doping on osteoclast functionality. PBMC-derived CD14+ cells were differentiated on pellets of each ceramic for 21 and 28 days. The results showed that multinucleated osteoclasts (detected by TRAP assays and $\alpha_V\beta_3$ integrin immunostaining) formed predominantly on CHA, whereas minimal osteoclastic differentiation was observed on the other ceramic compositions.

Conclusions

In summary, our study showed that carbonate doping enhances both dissolution and osteoclast-mediated degradation of the ceramic material, whereas silicate doping or co-doping does not significantly affect either process. Additionally, the *in vivo* degradation observed in CSiHA and β-TCP may be mediated by inflammatory cells other than osteoclasts, which remain to be identified.

Topic: Bioprinting & Biofabrication
Subtopic: 3D bioprinting and biofabrication
Type: Oral presentation

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TERMIS25_881 - Development of a Novel Biomaterial Ink & Ceramic Paste Reinforced with Bioactive Materials for Bone Tissue Engineering Applications

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Introduction/Objectives: Bone is a highly dynamic tissue that offers structural and mechanical support to the body. In general, large bone defects resulting from trauma, accidents, falls & sports injuries, typically require surgical intervention. Current clinical options for bone repair include autografts and allografts; however, these methods have several drawbacks, including limited graft availability, donor site morbidity and potential immune rejection. Tissue engineering has emerged as a promising alternative to overcome limitations associated with the above methods. Among various fabrication methods, 3D printing is emerging as a promising method because it offers precision in depositing biomaterial inks to make complex tissue structures and also enable the fabrication of bespoke scaffolds for specific defect sites in patients. Due to several advantages, ceramic materials such as hydroxyapatite has gained more attention in making bone scaffolds using 3D printing. Further, to improve the biological as well physicochemical properties, ceramic materials are combined with metal-organic frameworks (MOFs) and emerging materials like bentonite, a clay mineral enriched with elements such as SiO_2, Al, Mg, Na, K and trace amounts of iron to fabricate bone constructs. These materials offer several advantages in accelerating bone repair by enabling the delivery of biomolecules and therapeutic agents, promoting angiogenesis, and exhibiting antibacterial properties.

Methods: In this work, we have developed a novel ceramic paste (biomaterial ink for 3D printing) with a combinations of hydroxyapatite (HA)/MOFs and HA/bentonite. Firstly, different types of MOFs were synthesised using solvothermal techniques and were blended with HA and nano hydroxyapatite (nHA) in various ratios with appropriate binders to create ceramic pastes/biomaterial inks, which were then mould casted or 3D printed into various complex bony shapes.

Results: Following a high temperature sintering process, the mould-casted and 3D-printed scaffolds were examined for their structural, mechanical, and physicochemical characteristics using TGA, FTIR, XRD and XPS. The scaffolds retained their crystalline nature and metallic components in the form of metal phosphates and carbides, which eventually resulted in the increased mechanical strength of the developed composite scaffolds. *In vitro* studies conducted using MG63 (osteosarcoma) cell line revealed that both the mould casted & 3D printed scaffolds with various MOFs at different ratios were found to have excellent cytocompatibility and have also shown higher cell adhesion & proliferation when compared to the pristine HA & nHA groups.

Conclusions: In conclusion, the mould-casted and 3D-printed scaffolds demonstrated enhanced structural, mechanical, and physicochemical properties. The *in vitro* studies with MG63 cells highlighted the excellent cytocompatibility of both the mould-casted and 3D-printed scaffolds, showing increased cell adhesion and proliferation compared to pristine HA and nHA groups. Future *in vivo* studies in rat and rabbit models will provide valuable insights into the efficacy of these scaffolds for treating critical-size bone defects, both load-bearing and non-load-bearing. These findings suggest the promising potential of the developed scaffolds for advanced bone regeneration applications.

Topic: Biomaterials
Subtopic: Ceramics
Type: Oral presentation

TERMIS25_889 - Microporous bioactive glass scaffolds fabricated by two-photon lithography for in vitro applications

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Introduction/Objectives

Microstructured scaffolds made of bioactive materials, such as bioceramics or bioactive glass (BG), can improve 3D cell culture or even the performance of bone implants. Bioactive glass dissolves upon contact with body fluids and induces the precipitation of hydroxyapatite, which provides a favorable environment for cell growth and tissue attachment. Compared to ceramics such as hydroxyapatite, it degrades much faster and can be replaced by new tissue. Because of its high bioactivity, it is in clinical use for bone grafts and is a promising material for 3D cell culture. For these applications, the microstructure, especially the porosity, has a profound effect on the biological response. Pores larger than 100 μm allow cell invasion and metabolic transport. Even smaller pores of influence cell adhesion and proliferation. In addition, the differentiation behavior of stem cells can potentially be influenced by their geometric environment. However, current fabrication methods for inorganic biomaterials do not allow structuring below 100 μm with full design freedom.

Methods

Two-photon lithography (TPL) is a 3D printing technique capable of sub-micron resolution structuring. However, material development for TPL is challenging and has not been achieved for inorganic biomaterials. To address this problem, we developed a BG-containing nanocomposite that can be structured by TPL. We synthesized nanoparticles with the composition of 10 mol% CaO and 90 mol% SiO2 via a modified Stöber process. Our synthesis can be easily modified for other compositions that may contain therapeutic ions (e.g. B, Sr, Cu, Zn). The particles were dispersed in an acrylic binder to obtain a photocurable nanocomposite. The nanocomposite was structured by TPL, the organic content was subsequently removed at 400 °C, and the glass particles were densified by sintering at 900 °C.

Results

With our process, we achieved single-micron resolution for 3D BG structures for the first time. This allowed the fabrication of various high-resolution structures, such as octet and diamond lattices, with beams of 10 μm in diameter and pores between 12 and 50 μm. The scale-up potential was demonstrated by replicating a centimeter-sized cancellous bone scaffold. Such a scaffold size is essential for practical applications in cell culture as well as for potential use in implants. We demonstrated the cytocompatibility and investigated the differentiation behavior of human mesenchymal stromal cells on these scaffolds.

Conclusions

The high-resolution structuring of BG by TPL allows in-depth studies that investigate the influence of microstructure on cell behavior.

Free design freedom combined with single-micron resolution was achieved and centimeter-sized scaffolds, suitable for cell culture, were produced. The use of BG is particularly relevant for bone cells and other cells found in bone, including mesenchymal stromal and hematopoietic stem cells.

Topic: Biomaterials
Subtopic: Ceramics
Type: Oral presentation

TERMIS25_894 - PCL/P2O5-free bioactive glass composite scaffolds for enhanced osteogenesis and angiogenesis during bone regeneration

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Introduction/Objectives
Bioactive glasses (BGs) have emerged in bone tissue engineering due to their combined osteogenic and angiogenic properties. Particularly, P_2O_5-free BGs powders have been shown to possess high bioactivity *in vivo*. However, their incorporation in additive manufactured (AM) scaffolds was not reported before. Therefore, in this study, we fabricated 3D scaffolds made of composites of polycaprolactone (PCL) and two novel P_2O_5-free glasses with different trace ion composition, BG-WS and BG-YS, in a ratio PCL:BG of 80:20 w/w. We assessed the osteogenic and angiogenic potential of the constructs in an *in vitro* study with human mesenchymal stromal cells (hMSCs) and human umbilical vein endothelial cells (hUVECs).

Methods
Two composite materials were produced by incorporating a high content of either BG-WS or BG-YS (20% w/w) into a PCL matrix. Using melt-extrusion AM, we fabricated scaffolds with star-shaped pore geometries. These scaffolds were then treated with NaOH to partially degrade the superficial layer of the fibers. Subsequently, scaffold degradation and release of Si and Ca ions were investigated in both PBS and PBS supplemented with physiological lipase concentration (i.e. to mimic the *in vivo* environment). We assessed the mechanical performance of the scaffolds by compression tests. Then, we seeded hMSCs on both PCL only and composite scaffolds and cultured them for up to 28 days. The expression of several osteogenic markers and protein secretion from hMSCs were investigated via qPCR, biochemical assays, and multiplex analysis. Finally, hUVECs were cultured in conditioned medium (CM) from hMSCs collected at day 7 (CM7) and day 28 (CM28) to evaluate the angiogenic potential of the scaffolds indirectly.

Results
Scanning electron microscopy (SEM) images confirmed the successful fabrication of the scaffolds and revealed that NaOH treatment exposed BG particles on the scaffold surface.

Moreover, the addition of the BG particles mechanically reinforced the PCL matrix, leading to a higher compressive modulus than in the PCL scaffolds (49.40 ± 2.95 MPa vs 18.67 ± 1.51 MPa). In PBS, the release of Si and Ca ions after 28 days reached up to 50 and 4 ppm, respectively. The addition of lipase to the solution further accelerated the release as result of increased scaffold degradation. After confirming release of ions, we assessed the osteogenic and angiogenic potential of the scaffolds. Both composite scaffolds, but especially BG-WS, stimulated the upregulation of hMSCs osteogenic genes, such as RUNX2, COLL1A1, and OCN, compared to PCL only scaffolds. Additionally, composite scaffolds promoted both higher VEGFA gene expression and protein secretion after 28 days. Lastly, a tubule formation assay with hUVECs cultured in CM7 and CM28 was used to indirectly assess the scaffold's angiogenic properties. Cells exposed to CM28 from the composite scaffolds displayed significantly larger mesh size compared to those treated with medium from PCL scaffolds.

Conclusions
Overall, our data support the hypothesis that P_2O_5-free bioactive glass-based composite AM scaffolds have great potential in promoting both osteogenesis and angiogenesis, key processes in successful bone tissue regeneration. Future research will be directed towards *in vivo* studies to further confirm these findings in clinically relevant animal models.

Topic: Biomaterials
Subtopic: Ceramics
Type: Oral presentation

TERMIS25_1122 - Comprehensive evaluation of 3D-printed hyperelastic PLA-TCP (60:40) scaffolds: mechanical, thermal, and morphological properties upon immersion in SBF

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Introduction/Objectives
The use of commercial medical-grade biomaterials is an essential requirement for moving a lab-based technology to a higher Technology Readiness Levels. Over the past decade, a greater range of Good Manufacturing Practice-manufactured biomaterials of different formulations have become available, including material in

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filament form specifically for Fused Deposition Modelling (FDM) 3D printing.

The present study aims to characterise the physicochemical properties of 3D printed scaffolds manufactured using filament made of a medical-grade 60/40 blend of Lactoprene® 7415 and β-tricalcium phosphate. Lactoprene® is a linear segmented copolymer, consisting of 74% lactide, 15% trimethylene carbonate, and 11% ε-caprolatone monomers.

Methods
This study optimised the printing process to improve layer bonding and minimise voids, producing solid samples that accurately represent the bulk material properties. Once fabricated, the samples were subjected to uniaxial compression testing, following ASTM D695-23 standard, in dry and aqueous media to study the effect of water molecules and temperature on composite mechanical properties. Tests were performed at ambient (25 °C) and 37 °C simulated physiological conditions. The extent of elastic recovery and water absorption of the material were also assessed. FTIR analysis explored the molecular interactions between the composite material and water molecules, while DSC was employed to study the alteration in thermal characteristics.

Results
The results showed that the mechanical properties of the composite were significantly impacted when exposed to simulated physiological conditions. Under ambient conditions the composite compressive performance is typical of a semi-crystalline polymer, however at simulated physiological conditions the profile changes significantly to one representing a highly elastic material. This is followed by a 74% improvement in recovery behaviour/shape memory when the test condition changes from dry ambient to the simulated physiological conditions. The study also revealed that water molecules can induce plasticization by modifying the thermal and chemical properties under physiological conditions, compared to ambient conditions.

Conclusions
The composites exhibit advantageous physico-chemical properties for scaffold-guided bone regeneration, together with a high ceramic content that could benefit the biological performances in future in vivo assessments. Such scaffolds are, therefore, promising candidates for bone tissue engineering.

Topic: Biomaterials
Subtopic: Ceramics
Type: Oral presentation

TERMIS25_1388 - 3D Printable Calcium Sulphate Based bone void fillers for Localised Drug Delivery

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Introduction/Objectives
Osteomyelitis whether haematogenous or due to direct inoculation in open fractures is critical to be addressed at the earliest. Standard treatment with prolonged systemic/intravenous antibiotics can adversely affect gut health, renal and hepatic functions, impacting overall quality of life. Moreover, prolonged antibiotic exposure increases the risk of antimicrobial resistance. Localised drug delivery, specifically α-hemihydrate, crystalline $CaSO_4$ has demonstrated enhanced attributes of fast setting, high initial strength and complete resorption. Surgical debridement of the necrotized tissue leads to formation of complex voids with irregular shapes. Here, we intend to develop injectable (powder + gel) bioink formulation able to carry drug and provide localised delivery. The speciality of the injectable to express controlled viscosity with temperature tunning (inverse thermos responsiveness of liquid (poloxamer) portion), aids in smoother injectability, lesser force of injection and complete filling of complex voids.

Methods
In this study we were able to successfully synthesise short column crystals, with Citric acid as crystal modifier. The product was characterised with **XRD** for crystal and phase analysis and **FTIR** to identify any residual solvents. Scanning electron microscopy (**SEM**) provided info about crystal morphology. Thermogravimetric (**TGA**) analysis was done to quantify water content. Further setting time (**Gillmore needle**) and compressive strength (UTM) were analysed as per **ISO/DIS 18531:2015(E).** Workability of the powder was assessed based on its ability to be drawn into thin sheets. The powder and injectable were both tested for **MTT** cytotoxicity on L929 cell lines as per **ISO 10993 5&12**. the Inverse thermoresponsive (IVT) was determined by **Rheological** and **Injectability** testing at temperature range from 10 °C to 40 °C. This formulation was loaded into luer lock syringe and subject to **3D Bioprinting.**

Results
Signature peaks from XRD confirmed successful synthesis of hemihydrate and SEM micrographs demonstrated short crystal morphology – validating the α-HH. FTIR proved no presence if solvent implying proper drying of powder. TGA showed excellent purity of 99.2% of HH formation with an indicative 6.245% water loss. The powder was mixed with DI water in l/p ratio of 0.3, demonstrating an initial setting time of < 3 mins and final setting time of < 7mins. Testing of 6x10 mm cylindrical specimen yield an average compressive strength of 23 MPa. The α-HH and DI paste at 0.3 l/p ratio demonstrated excellent workability to drawn in sheets <1mm thick. Rheological data confirmed the IVT nature of the injectable which showed gelling phenomenon at 27°C, which also reflected in minimal residual volume of < 0.3 ml in a 6 ml syringe (95% injectability). Additionally, the reduced viscosity allowed the formulation to be extruded through a finer 20G needle onto a heated plate maintained at 35°C, which resulted in better printing resolution. MTT cytotoxicity testing on the L929 cell line demonstrated an acceptable Grade I level, indicating the formulation's safety.

Conclusions
The synthesised α-HH showed superior strength at minimal water requirement. The short column morphology enhanced injectability and IVT property of poloxamer comprehensively aids in better injectability. This formulation demonstrated both capability of complete void filling and 3D printability.

Topic: Biomaterials
Subtopic: Ceramics
Type: Oral presentation

TERMIS25_627 - Characterization of an in vivo critical-sized bone defect regeneration using bioactively coated degradable ceramic granules

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Introduction/Objectives
Bone autografts are the gold standard for large bone defect treatments. However, their sources and amount are limited. Thus, large bone defect repair strategies are in need of new alternatives to overcome these restraints. With this objective, we have developed a combinatorial approach employing degradable ceramic bone graft granules (monetites), coated with a highly bioactive polymer, poly(ethyl acrylate) (PEA), which induces the unfolding of fibronectin

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(FN), increasing the efficiency of bone morphogenetic protein-2 (rhBMP-2) [1].

Methods
To analyse the efficacy of the system, two monetites (2.5x1.5mm) were implanted in a critical 5-mm defect in rat femora. Monetites were either plain or coated with PEA, FN and/or rhBMP-2 in different concentrations (10 and 25 μg/ml). Platelet-rich plasma (PRP) was also investigated as an alternative to FN. These coatings were used on their own or in different combinations. A multiscale characterization of non-decalcified explants embedded in poly(methyl methacrylate) (PMMA) was performed ten weeks after implantation. This was implemented using high-resolution microcomputed tomography (microCT), controlled angle cutting [2], scanning electron microscopy (SEM) in back-scattered electron (BSE) mode, histological staining and polarized light imaging, and histomorphometric analysis.

Results
First, microCT and 3D reconstruction analysis revealed the effectiveness of the PEA, FN and rhBMP-2 coating (in both concentrations) for bone regeneration, as only those coatings were capable of bridging the defect. Second, newly formed bone volume (BV) was quantified at (i) the immediate material-tissue interface and (ii) the whole defect total volume (BV/TV), using ORS Dragonfly software for microCT image analysis. Samples containing PEA and rhBMP-2 coated monetites showed a significant bone volume increase in both regions (p-values<0.05). On the other hand, PRP coating significantly reduced the bone formation both at the vicinity of the monetite and the whole defect volume. In addition, high-resolution microCT image quantification using the deep learning tools of ORS Dragonfly demonstrated that monetites degraded nearly 20% in volume and doubled the interconnected porosity after 10 weeks of orthotopic implantation, favouring new bone ingrowth. This was corroborated with BSE images, which showed seamless interfaces between monetite and new bone, with greater bone infiltration in the PEA+FN+rhBMP-2 coated grafts, where the osteocyte lacunae could be clearly identified. These infiltrations were not observed in uncoated or PRP-coated grafts. Finally, the histological sections provided information about the soft tissue surrounding the monetites. Movat pentachrome-von Kossa stainings, alcian blue and picrosirius red staining with polarized light imaging showed higher amounts of collagen and immature mineralized tissue in the animals with PEA+rhBMP-2 coated grafts. Last, tartrate-resistant acid phosphatase (TRAP) staining showed the presence of osteoclasts in the non-bridged samples, suggesting bone remodelling activity.

Conclusions
In conclusion, this work proved the efficacy of degradable ceramic granules coated with osteogenic compounds (PEA and low-dose rhBMP-2) for bone regeneration applications.
Acknowledgements: H2020-HEALIKICK project (874889).
[1] Cheng, Z.A., et al., 2019, Adv.Sci., 6, 1800361.
[2] Moreno-Jiménez, I., et al. 2021, MethodsX, 8, 101480.

Topic: Biomaterials
Subtopic: Fiber-based biomaterials
Type: Oral presentation

TERMIS25_1378 - Biomaterial scaffold architecture induces endochondral healing of bone defects beyond mechanobiological limits

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Introduction/Objectives
Until today, no pure biomaterial strategy for bone defect regeneration exists, even though associated low costs, off the shelf availability and a low risk of side effects are clear advantages. We have previously shown that a soft, collagen-based biomaterial with a channel-like pore architecture is able to induce development-like endochondral ossification (EO) for the healing of critical size bone defects (Petersen et al., Nat Commun 2018). Here, we report on the incorporation of a 3D printed support structure to improve the mechanical stiffness of the material and to understand the mechano-biological limits of scaffold-induced EO in respect to a clinical application.

Methods
A soft and a stiff version of the support structure were designed in SolidWorks (Dassault Systems) and produced from medical grade polycaprolactone (PCL) by selective laser sintering. Mechano-hybrid-scaffolds (MHS) were produced by introducing the support structures into a collagen dispersion before linear ice templating, freeze drying and crosslinking. Mechano-bioreactors were used to characterize cell recruitment into MHS and to analyze stiffness and fatique under in vivo-like loads. MHS were implanted into 5mm critical size defects in the rat femur while no bioactive molecules, cells or bone grafts were added. Bone defect healing was studied via x-ray, μ-CT and (immune)histology.

Results
The strong cell recruitment potential of the collagen-based guiding structure was not impaired in MHS. Stiff and soft MHS showed a mechanical stiffness of 72 ± 15 N/mm and 0.095 ± 0.021 N/mm, respectively and did not show material fatigue over >1*106 compression cycles. Predicted in vivo strains were 1.2% for stiff and 7.8% for soft supports. Implantation of MHS into the bone defect revealed a robust induction of endochondral ossification for soft but also for stiff MHS prototypes, despite the low respective tissue strains. Nine weeks after implantation, three out of six animals with stiff MHS were in the process of bony bridging as verified by μ-CT and histology.

Conclusions
The results of this study verify the potential of the architecturally optimized MHS to induce the regeneration of critical size bone defects via EO even at low mechanical strains found in situations of stiff bone fixation.

Topic: Submit to SYMPOSIUM
Subtopic: Clinical Translation in Bone Tissue Engineering
Type: Oral presentation

TERMIS25_1426 - Predicting endochondral bone regeneration capacity of engineered hypertrophic cartilage tissues

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Introduction/Objectives
Endochondral bone regeneration (EBR) offers a promising alternative to autografts for treating bone defects that do not heal spontaneously. This strategy recapitulates the natural process of callus formation in fracture healing by engineering cartilage templates in vitro, which are remodeled into bone tissue post-implantation. Typically, EBR employs chondrogenically differentiated mesenchymal stromal cells (MSCs) to create such cartilage templates in vitro. Despite its potential, EBR's clinical translation remains hindered by an incomplete understanding of how the expression of various differentiation characteristics is related to in vivo bone formation outcomes. Therefore, this study aims to control the in vitro cartilage differentiation status and to identify predictors of in vivo bone formation.

Methods
Human MSCs isolated from six donors underwent chondrogenic differentiation in spheroid culture (250,000 MSCs/spheroid) for 28 days using either TGFβ alone or TGFβ/BMP2 to tune matrix composition. Resulting cartilage tissues were analyzed histologically and biochemically to assess extracellular matrix composition, including collagens, glycosaminoglycans (GAGs) and various differentiation marker proteins.

Furthermore, an ongoing in vivo study involves implanting these cartilage spheroids subcutaneously in immunocompromised rats. After two months, bone formation will be evaluated using microCT scanning to measure mineralization volume and histological analysis.

Results
Preliminary results indicate that BMP2 supplementation enhances chondrogenic matrix deposition, as evidenced by increased spheroid size, GAG content, and more intense Safranin O and collagen type II staining. Additionally, BMP2 supplementation promoted a hypertrophic phenotype characterized by elevated alkaline phosphatase (ALP) activity and more intense collagen X staining. BMP2-treated spheroids also exhibited increased VEGF and BMP2 content. These effects were observed across all donors but were pronounced in those with limited chondrogenic potential when exposed to TGFβ alone. This suggests that BMP2 supplementation not only enhances matrix production in donors with high chondrogenic potential but also rescues poor differentiation outcomes in low-performing donors. Critically, the observed increases in ALP activity, VEGF content, and BMP2 levels suggest that BMP2 supplementation may enhance the bone-forming potential of these spheroids. The ongoing in vivo study will determine whether these markers correlate with bone formation and whether additional predictors of in vivo performance can be identified.

Conclusions
In conclusion, this study highlights BMP2's potential to improve hypertrophic chondrogenic differentiation for EBR. By identifying key in vitro characteristics and correlating these with in vivo bone formation outcomes, this research aims to identify release criteria for clinical translation of EBR-based therapies. Such advancements could enhance EBR's clinical utility by enabling high-quality cartilage implant production for effective bone defect treatment.

Topic: Cells
Subtopic: Cell niche
Type: Oral presentation

TERMIS25_977 - Peptide-based niche engineering for selecting mesenchymal stem cells with different glycan profiles

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Introduction/Objectives
Mesenchymal stem cells (MSCs) are valued for their multipotency and immunosuppressive properties, making them vital for advanced therapy medicinal products. However, maintaining MSC quality during *in vitro* culture remains challenging. *In vitro*, the properties of culture surfaces can bias the MSC populations, emphasizing the need for ideal culture interface designs. Recent studies suggest that glycan profiles can be new markers on the MSC surfaces which reflect subtle quality changes. This study aims to design cell culture surfaces that selectively promote the adhesion and growth of MSCs which maintain high-quality glycan profiles. By screening extracellular matrix (ECM)-derived short peptides, we sought to develop culture substrates capable of distinguishing between MSCs with different glycan profiles.

Methods
Human bone marrow-derived MSCs were cultured in MSC-GM medium, with three cell lots passaged intentionally from passage 2 to passage 6. Glycan profiles of these lines were analyzed using a lectin array, revealing consistent changes in surface glycans with extended passages. A library of 80 ECM-derived tripeptides was used to create peptide-coated arrays, and MSC adhesion and spreading rates were measured by image analysis to identify peptides that selectively support high-quality MSCs.

Results
Lectin array analysis confirmed distinct glycan profiles between low-passage and high-passage MSCs. Glycan-specific lectin-conjugated nanoparticles further validated these differences. Screening of the peptide-coated arrays revealed that MSCs with different glycan profiles exhibited significant differences in adhesion and spreading rates on specific peptide sequences.

Conclusions
This study demonstrated the potential of peptide-coated interfaces to selectively culture MSCs based on glycan profile-related quality changes. Additionally, MSCs with distinct glycan profiles showed differences in phagocytic activity and differentiation potential, underscoring the importance of glycan profiles in MSC performance evaluation. These findings pave the way for glycan-targeted quality control and optimization of MSC culture systems.

Topic: Biomaterials
Subtopic: Micro-&nanoscale materials
Type: Oral presentation

TERMIS25_1363 - Controlling and predicting the osteogenic phenotype by implant surface topography

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Objectives
Former work demonstrated that microtopographies of titanium and ceramic implants control osteoblast morphology and subsequent cell behavior such as mitotic activity. Thus, we hypothesized that topographical implant surface features can be used to control and predict osteoblast phenotype through cell morphology.

Methods
We investigated whether surface topography data combined with quantitative single cell morphology and gene expression analysis can be used to first establish predictive models describing the relationship between biomaterial surface features, cell morphology and osteogenic gene expression. Second, we aimed to identify key implant features that determine the cell morphology and function that are relevant for bone formation by human primary osteoblasts. A panel of topography parameters of 9 clinically established implant biomaterials with different topographies was quantitatively analyzed by white light interferometry. The shape of primary human osteoblasts (7 donors) cultured for 1 and 7 days on the different implant surfaces was analyzed by image-based quantitative morphometry using a panel of shape descriptors (area, perimeter, aspect ratio, roundness, circularity). The expression profiles of osteogenic markers were quantified by Droplet Digital PCR. Statistical analysis, multivariate data exploration, and projection-based modelling were used for establishing predictive models for the relationship between implant surface topography, cell morphology and osteogenic gene expression, and, second, identifying key implant surface features that control the osteogenic phenotype.

Results
Our data demonstrated significant correlations between cell area-related shape descriptors and topography parameters describing amplitude height and spatial distribution of the surface features. Biomaterials with rough surface characteristics induced smaller and rounder cell morphologies than smoother biomaterial surfaces at day 1. Interestingly, this relationship between surface topography and cell morphology persisted until day 7. Among the examined

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osteogenic genes, BMP2 showed a significant positive correlation with the above-described topographical surface parameters at both culture time points. Regression analysis using values of the surface roughness parameter Sa, cell size and BMP2 expression revealed that a logistic regression model ($p<0.05$) well-described individual cell size on a given biomaterial with known surface roughness values Sa ($R^2 = 0.866 - 0.946$). In contrast, linear regression was more suitable to describe the relationship between surface roughness Sa and BMP2 expression. Projection-based modelling further identified height and hybrid surface parameters as important features for the modulation of the cell area and BMP2 expression.

Conclusions
Implant microtopography parameters describing the surface roughness and other characteristics of surface features can be used to control the osteogenic phenotype of human osteoblasts on implant surfaces. Moreover, the interplay between quantitative surface topography, morphometry and gene expression analysis in conjunction with advanced methods for multivariate data analysis allows identifying key implant features for predicting cell behavior at the tissue-biomaterial interface. This approach could be used to design implants with defined surface structures, supporting a long-term integration of biomaterials into the target tissues.

Topic: Cells
Subtopic: Cell niche
Type: Oral presentation

TERMIS25_1384 - Bioengineered niches that recreate physiological bone marrow extracellular matrix organization to support long-term hematopoietic stem cells, model CAR T cell therapy, and support cancer remodeling

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Introduction/Objectives
The bone marrow niche is a highly specialized microenvironment that regulates haematopoietic stem cell (HSC) self-renewal and differentiation. It consists of a low-stiffness, hydrogel-like extracellular matrix and HSC-supporting mesenchymal stromal cells (MSCs). HSCs are critical for treating blood disorders through stem cell transplantation; however, their clinical application is limited by their low abundance in bone marrow and their rapid differentiation during in vitro culture. Furthermore, in diseases of the bone marrow, such as leukaemia, MSCs drive niche remodelling to support cancer growth. In this work, we develop bioengineered models of the bone marrow microenvironment that mimic its mechanical properties to support niche-like phenotypes in MSCs. These models are used to investigate HSC maintenance[1], MSC-driven cancer remodelling during cancer evolution, and as a platform for testing chimeric antigen receptor (CAR) T cell therapies.

Methods
We report bioengineered bone marrow niches that recreate physiological extracellular matrix organization and mechanical properties. Poly(ethyl acrylate) (PEA) surfaces are synthesized to self-organize the extracellular matrix protein fibronectin (FN), exposing FNs growth factor-binding domain[2]. We adsorb ultra-low concentrations (50 ng/mL) of bone marrow-related factos such as BMP-2, CXCL12, and NGF. Low-stiffness properties of the native bone marrow are introduced by overlaying biological (collagen type-I), synthetic (peptigels), or synthetic-biological hybrid hydrogels (PEG-FN).

MSCs were characterized for niche phenotypes using next-generation sequencing (NGS), flow cytometry, and fluorescence microscopy. The niches' capacity to support HSCs in culture was evaluated through flow cytometry and functional assays, including long-term culture-initiating cell (LTC-IC), colony-forming unit (CFU) assays, and in vivo reconstitution studies. Leukemic cells and CAR T cells were introduced to study niche remodeling during cancer evolution and to assess potential therapeutic strategies.

Results
We demonstrate that low-stiffness environments promote niche-like phenotypes in MSCs (e.g. nestin+ MSCs, production of HSC maintenance factors), capable of supporting long-term HSCs in culture[1]. When leukaemic cells were introduced, we show an increase in nestin expression in the leukaemic stroma, that is validated against primary patient samples. Furthermore, we demonstrate a novel leukemia treatment strategy involving CRISPR gene editing of HSCs to protect them from ablation by leukemic-targeting CAR T cells.

Conclusions
Mechanical properties, such as low-stiffness microenvironments, support key niche phenotypes in MSCs that can support LT-HSCs in culture. We demonstrate the use of BM platforms to uncover mechanisms involved in healthy niches and cancer remodeling of the bone marrow, validated against clinical observations. Finally, we show the development of a BM microenvironment platform to test novel leukemia therapies on human cells in vitro.

1. Donnelly et al., Nature Communications (2024)
2. Cheng et al., Advanced Science (2018)

Topic: Tissue Engineering
Subtopic: Tissue modelling and repair
Type: Oral presentation

TERMIS25_1532 - Precise femtosecond laser-micropatterning of 3D topographical platforms for the modulation of cellular fate

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Introduction/Objectives
Cell-substrate interactions are vital to mimic the *in vivo* microenvironment and stablish rational design criteria for scaffold manufacturing. The intrinsic properties of the extracellular matrix (geometry, porosity, topography) influence cell behavior through biochemical pathways. Although the influence of topography has been studied in 2D substrates, the complexity of the existing methodologies hinders the translation of these findings to 3D. Laser ablation techniques allow the introduction of precise superficial micropatterns without the use of chemicals or sacrificial materials, representing an insightful approach to the sustainable fabrication of topographically modified devices. The objectives of this work were: 1) the development of an innovative additive-subtractive technique by integrating 3D printing and laser ablation, allowing for the manufacturing of scaffolds with precise microtopography without the limitations of the current methodologies; 2) the structural characterization of the micropatterned scaffolds to assess overall feasibility; and 3) the *in vitro* testing of the scaffolds to determine their effects on cell fate.

Methods
Scaffold fabrication: Poly-(ε-caprolactone) scaffolds were designed with three distinct AutoCAD patterns: 10 or 80 μm microgrooves, and 25 μm-diameter micropits (PCL-G10, PCL-G80, and PCL-MP, respectively). A 3D extrusion printer and a femtosecond laser were used for manufacturing.

In vitro assays: PCL-G10, PCL-G80, PCL-MP and controls were dynamically seeded in syringes with 1 mL of human mesenchymal stem cells suspension and culture media. On days 1, 3 and 7, scaffolds were washed, fixated with 4% formaldehyde, blocked and incubated with 4',6-diamine-2'-phenylindole and phalloidin-Alexa Fluor 488. Fluorescence results were obtained with confocal microscopy. Nuclear counting, morphological analysis, and orientation angles were measured using an image analysis software.

Results
Structural characterization: Scanning electron microscopy and microcomputed tomography revealed that scaffolds maintained their

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overall integrity after ablation. Micropatterns exhibited great fidelity with respect to the original design, maintaining alignment along the central axis of the fiber. Femtosecond laser ablation permitted PCL to be easily expelled, with a good micropattern resolution and no debris or redeposition. Patterns needing the highest power intensity (PCL-G80, PCL-MP) reproduced the Gaussian footprint in their cross section.

hMSCs behavior: PCL-G10 exhibited a significantly higher attachment than the remaining conditions, presumably because the 10 μm width-range facilitated the formation of focal adhesion complexes. Proliferation studies and cytoplasm analysis revealed that microgrooved scaffolds favored cellular proliferation on day 1, and greater alignment over time.

Conclusions
The integration of 3D printing and femtosecond laser ablation enabled the manufacturing of scaffolds with precise, reproducible micropatterns on all layers of the device, overcoming precedent limitations regarding low surface accessibility, loss of micropattern resolution, or debris accumulation whilst preserving the original properties of the polymer. Besides, the *in vitro* evaluation confirmed that pattern dimension and geometry increased hMSCs alignment and alignment, exhibiting great potential as 3D platforms for studying the effects of topography on cell fate.

Topic: Submit to SYMPOSIUM
Subtopic: Designing advanced bioinspired materials by merging natural macromolecules with supramolecular chemistry
Type: Oral presentation

TERMIS25_447 - Biofabrication of injectable granular matrices for cell delivery through the supramolecular assembly of decellularized human tissue

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Introduction/Objectives
Motivated by the limited survival and retention of cells delivered to wounded tissues, the development of injectable matrices that can retain cells at the site of injection has become an active field of research. Seeking to develop materials that can better preserve cell viability and retention while facilitating integration with native tissue, decellularized extracellular matrices (ECM) derived from tissues of human origin can be exploited as a potential source of new biomaterials for cell delivery. By coupling the use of these materials with supramolecular assembly strategies, we aim to reproduce the biophysical characteristics of native ECM in our scaffolds.

Methods
The host-guest complexation of human amniotic membrane (hAM) with a photopolymerizable cyclodextrin was used to assemble soft viscoelastic hydrogels. Potential changes to the secondary structure of hAM proteins were assessed through circular dichroism and Fourier-Transform Infrared (FTIR) Spectroscopy. Through extrusion fragmentation of the initial hydrogels, self-curing injectable granular matrices were obtained. Granular materials were mechanically characterized through rheological analysis and compression testing. Biological performance was assessed by co-injection of microgranules alongside human adipose-derived stem cells (ASCs), and through *in vivo* experiments in a chicken (*Gallus gallus*) embryo model.

Results
The secondary structure of hAM proteins was preserved after hydrogel formation. Fragmentation of the supramolecular hydrogels yielded microgranules with extensive self-healing capabilities, able to reconstitute into bulk scaffolds that adapt to the morphological characteristics of the surrounding environment. The granular matrices were found to be shear-thinning, requiring reduced injection forces to allow their extrusion, and thixotropic, exhibiting rapid recovery after injection. Cells injected alongside the microgranules were able to adhere to their surface and exhibited extensive spreading behavior, achieving over 80% cell viability after injection. When applied in an *in vivo* model, the granular hydrogels displayed pro-angiogenic properties.

Conclusions
The supramolecular assembly of decellularized human ECM allows the generation of scaffolds with close-to-native structural and biomechanical properties. By processing these scaffolds into injectable matrices, it is possible to preserve cell viability during injection, and potentially beyond it, by enhancing cell retention through adhesion to the microgranules, and by promoting the recruitment of nascent blood vessels.

Topic: Tissue-Specific Focus
Subtopic: Angiogenesis, vasculogenesis
Type: Oral presentation

TERMIS25_689 - A Chromatin Signature by the Methyltransferase SETD7 Orchestrates Angiogenic Response in Diabetic Limb Ischemia

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Introduction: Peripheral artery disease (PAD) is highly prevalent in patients with diabetes (DM) and associates with a high rate of limb amputation and poor prognosis. Surgical and catheter-based revascularization have failed to improve outcome in DM patients with PAD. Hence, a need exists to develop new treatment strategies able to promote blood vessel growth in this setting. Mono-methylation of histone 3 at lysine 4 (H3K4me1) - a specific epigenetic signature induced by the histone methyltransferase SETD7 - favours an open chromatin thus enabling gene transcription. **Objective:** To investigate whether SETD7-dependent epigenetic changes modulate angiogenic response in diabetes. **Methods:** Primary human aortic endothelial cells (HAECs) were exposed to normal glucose (NG, 5 mM) or high glucose (HG, 25 mM) concentrations for 48 hours. Unbiased gene expression profiling was performed by RNA sequencing (RNA-seq) followed by Ingenuity Pathway Analysis (IPA). In vitro assays, namely cell migration and tube formation were employed to study angiogenic properties in HAECs. SETD7 and H3K4me1 levels were investigated by Western blot and Chromatin immunoprecipitation (ChIP). Pharmacological blockade of SETD7 was achieved by using the highly selective inhibitor (R)-PFI-2. Mice with streptozotocin-induced diabetes were orally treated with (R)-PFI-2 or vehicle and underwent hindlimb ischemia by femoral artery ligation for 14 days. Blood flow recovery was analysed at 30 minutes, 7 and 14 days by laser Doppler imaging. Our experimental findings were also translated in gastrocnemius muscle samples from patients with and without diabetes. **Results:** RNA-seq in HG-treated HAECs revealed a profound upregulation of the methyltransferase SETD7, an enzyme involved in mono-methylation of lysine 4 at histone 3 (H3K4me1). SETD7 upregulation in HG-treated HAECs was associated with increased H3K4me1 levels as well as with impaired endothelial cell migration and tube formation. Both SETD7 gene silencing and pharmacological inhibition by (R)PFI-2 rescued hyperglycemia-induced impairment of HAECs migration and tube formation, while SETD7 overexpression blunted the angiogenic response. RNA-seq and ChIP assays showed that SETD7-dependent H3K4me1 regulates the transcription of the angiogenesis inhibitor semaphorin-3G (SEMA-3G). Moreover, SEMA-3G overexpression blunted migration and tube formation in SETD7-depleted HAECs. In diabetic mice with hindlimb ischemia, treatment with (R)-PFI-2 improved limb vascularization and perfusion as compared to vehicle. Finally, SETD7/SEMA3G axis was upregulated in muscle specimens from T2D patients as compared to controls. **Conclusion:** Targeting SETD7 represents a novel epigenetic-based therapy to boost neovascularization in diabetic patients with PAD.

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Topic: Bioprinting & Biofabrication
Subtopic: Electrospinning, nanofiber spinning
Type: Oral presentation

TERMIS25_200 - Autopilot single-jet electrospun PCL-hydrogel hybrids for scalable and clinically relevant tissue engineering constructs

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Introduction/Objectives
In recent years, hybrid scaffold fabrication strategies have gained momentum in Tissue Engineering and Regenerative Medicine (TERM) field. 3D bioprinting (3DBP), which uses cell-suspended bioinks, enables the creation of hydrogel-based 3D cellular constructs. However, 3DBP faces critical challenges in scalability and structural resiliency due to the mechanical delicacy of hydrogels, where cell spreading and stability depend largely on hydrogel stiffness. This leads to a tradeoff between scalability, structural integrity, and biological activity. Our recent work demonstrated anatomically mimicking 3D polycaprolactone (PCL) electrospun scaffolds using autopilot single-jet 3D electrospinning (AJ-3D ES), which creates constructs with gradient porosity, high flexibility, shape memory, and exceptional mechanical properties. In this study, we advance this approach by integrating electrospun fibers with hydrogels, achieving two primary objectives: enhancing the biological functionality of AJ-3D ES PCL scaffolds and creating solid, thick hybrid constructs.

Methods
Our methods are divided into three parts: 1, **Bioink dip-coating**, in which PCL scaffolds are dipped into cell-suspended sodium alginate (SA) or gelatin methacrylate (GelMA) bioinks (up to 10 million cells/mL), followed by crosslinking ($CaCl_2$ or UV); 2, **Solid, thick construct fabrication** via techniques like bioink casting, freeform embedded reversible suspended hydrogels (FRESH) pocket 3DBP, or modular assembly, transforming topographic PCL scaffolds into thick 3D electrospun fiber-hydrogel hybrid tissue constructs; 3, **Robotic arm-integrated contour 3DBP**, e.g. vascular networks, onto 3D topographic PCL scaffolds for patient-specific tissue constructs in reconstructive surgeries.

Results
The hybrid constructs formed via bioink dip-coating showed accelerated tissue formation due to high cell loading within fibrous networks upon bioink crosslinking. While cells embedded within the hydrogel between fibers showed spherical morphology, cells attached to fibers proliferated and migrated, achieving high confluency. The porous fibrous network facilitated cell-cell interactions, resulting in cellular 3D self-assembly for diverse cell types, including MDCK epithelial cells morphogenesis, 3T3 fibroblast ECM remodeling, highly aligned myotube formation, and self-assembly of co-cultured HepG2 hepatocytes and HUVECs, mimicking liver tissue. Solid, thick hybrid constructs, where robust PCL scaffolds served as substrates, enhanced scalability, stability, and functional outcomes of hydrogel-based bioinks. Advanced integration, such as 3DBP, enabled the formation of anatomical constructs replicating target tissue composition at high resolution.

Conclusions
Unlike traditional 3DBP models, which rely heavily on hydrogel properties for shape, scalability, and stability, our work demonstrates that AJ-3D ES-fabricated PCL scaffolds can assume these critical roles in hybrid constructs. By shifting structural reliance from hydrogel characteristics to robust fibrous scaffolds, our study introduces scalable TERM constructs, up to human-organ size, that retain biological functionality without the quick degradation-associated loss of integrity typical of hydrogel-based models. As a result, these hybrid constructs have immediate clinical implications in diverse TE applications, beyond wound healing and reconstructive surgeries.

Topic: Biomaterials
Subtopic: Fiber-based biomaterials
Type: Oral presentation

TERMIS25_384 - Development of a functionalised Tendon Repair Augmentation Device using electrospun nanofibres

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Introduction/Objectives: Tendons are one of the key connective tissues within the human body and are responsible for transferring the forces exerted by muscles to bones to facilitate movement. Their functionality controlling high mechanical loads predisposes tendons as a key site of injury, with common tendinopathies including tendonitis, tendinosis, tears, and complete tendon rupture. Current therapeutic approaches include surgical intervention via tendon retrieval and reapproximation, with varying degrees of success dependent upon the complexity of the anatomical region. Limitations to the surgical practices include adhesion formation and insufficient mechanical capability restoration, increasing the risk of re-rupture post-surgery. Research efforts aim to mitigate the risk of re-rupture by developing biocompatible, functionalised materials to support tissue regeneration.

The established Tendon Repair Augmentation Device (TRAD) is a polymer-based, knitted scaffold that provides a biological niche to encourage cell in-growth to promote tissue regeneration at the injury site. This research aims to functionalise the established TRAD to deliver a controlled release of chemotactic biologics at the injury site to enhance cell infiltration, subsequently supporting healing further.

Methods: This research investigates the chemotactic effects of two growth factors (A & B) upon fibroblasts (L929) via Transwell Migration Assays over 12-hours. Assessments of cell proliferation were used to support evidence of migratory cell behavior using growth factor supplemented media. Manufacture of the functionalised material was conducted via differing electrospinning practices and confirmation of successful incorporation of biologics was assessed using a fluorescent protein conjugate as a biomimetic marker of protein integration. Further cell culture assays and immunofluorescence investigations were adopted to assess biocompatibility, cell viability and extracellular matrix (ECM) production in both 2D and 3D (electrospun mat, yarn and knitted product) environments.

Results: Preliminary studies indicate that the growth factors elicit chemotactic effects upon cell cultures, initiating cell migration over 12-hours. The electrospinning practices that were adopted to manufacture the functionalised material provide promise in successful protein incorporation within the material, as suggested by evidence using the fluorescent protein conjugate. Preliminary cell culture-based investigations suggest that the material is biocompatible and promotes successful cell adhesion and ECM production onto the 3D material constructs.

Conclusions: Initial investigations provide promise in the selection of chemotactic biologics and material manufacture techniques. Cell culture-based investigations suggest that the functionalised material is biocompatible and supports ECM production that is integral in tissue regeneration at the injury site. Future research aims to establish both elution and degradation profiles of the functionalised material. This research will provide the basis for cell migration studies, utilising live cell tracking to determine cell migratory behaviour in response to the elution of biologics from the functionalised material. Further investigation into the surgical delivery and mechanical capabilities of the developed material in porcine tendon tissue will be conducted.

Topic: Biomaterials
Subtopic: Fiber-based biomaterials
Type: Oral presentation

TERMIS25_400 - In situ crosslinked fibrous matrix-enhanced cell sheet engineering for improved cell interaction and targeted differentiation

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Introduction/Objectives
Owing to its similar structure to the extracellular matrix, electrospun nanofibers have been extensively investigated as bioscaffolds for cell culture. However, because it was hard for cells to move, a fragmented nanofiber called "nanofibril" (NF) was created to support cells to move freely compared to regular nanofibers. This also helps cells invade and interact with the matrix. We successfully employed cell-NF complexes in the treatment of osteochondral defects, peripheral nerve, and dermal wound. However, the poor mechanical properties of these complexes posed challenges during implantation process, and their destruction could potentially disrupt certain cellular biological expressions. Tetrazine (Tz) and trans-cyclooctene (TCO) have been studied as a powerful click reaction to address this challenge, enhancing the integrity of the cell-NF complex and cellular behaviors.

Methods
Tz and TCO were separately conjugated with multi-arm polyethylene glycol (PEG) using amide coupling reaction between PEG-4COOH and Tz-NH_2 (PEG-3Tz), and PEG-8NH_2 and TCO-NHS (PEG-8TCO). PEG-3Tz was further decorated on aminolyzed poly(ε-caprolactone) nanofibrils (PCL NFs). For *in vitro* studies, Tz@NFs were cultivated with human corneal epithelial cells (hCEC), and PEG-8TCO was added to generate the click reaction. To support hCEC differentiation, Tz-conjugated hyaluronic acid (HA-Tz) was supplemented after hCEC-NF complex was formed, so that HA-Tz could react with the remained free TCO moieties on the complexes. To investigate the mechanical property, the complexes were orbitally shaken at 250 rpm for 15 min, then the remained DNA content and weight were quantified. Cell distribution, cell viability, and DNA content of hCECs cultivated without NF in non-crosslinked and crosslinked complexes with native HA or HA-Tz were analyzed after 1 and 3 days of cultivation, and the expression of corneum-specific genes (keratin3, keratin12, PAX6, ALDH3A1, CD44) were evaluated by qRT-PCR after 3 days of cultivation.

Results
Tz and TCO were successfully modified on multi-arm PEG with the DS of 79.3% and 74.6%, respectively, which were confirmed by 1H NMR peaks at 7.6 and 8.4 ppm for Tz, and 5.5 ppm for TCO. After grafting PEG-3Tz on PCL NFs, the remained amine groups of Tz@NFs significantly decreased by 94.26 ± 5.43%, suggesting that PEG-3Tz was successfully decorated. After the click reaction between Tz@NFs and PEG-8TCO, the C-O stretching and N-H bending peaks at 1640 and 1530 cm^{-1} of the crosslinked NFs were clearly appeared on the FT-IR spectra. The addition of PEG-8TCO at the beginning step of hCEC-Tz@NFs complex cultivation with molar ratio of Tz-TCO = 1-1 showed the highest mechanical property among all conditions, which was confirmed by the negligible change of morphology after shaking, higher remained weight and DNA content. Cell was more homogeneously distributed in the earlier crosslinked complexes. While cell viability and DNA content was similar in all groups, the expression levels of specific genes of hCEC cultivated in crosslinked complex supplemented with HA-Tz were the highest among all groups.

Conclusions
The crosslink between Tz and TCO facilitated better integrity of the cell-NF complex, which enhanced its mechanical property. The click reaction also provided stronger HA binding on the complex, which effectively assisted cell differentiation.

Topic: Bioprinting & Biofabrication
Subtopic: Electrospinning, nanofiber spinning
Type: Oral presentation

TERMIS25_1071 - Exploring Electrohydrodynamics based Functional Nanofibers as Three-dimensional Nano-Biointerfaces

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Introduction/Objectives
The significance of the overall fibrillar and porous nanoscale topography of the extracellular matrix in promoting essential cellular processes has led to consideration of biomaterials with nanofibrous features. Of the many methods for fabricating fibers with micrometer and nanometer diameters, electrospinning is simplest, most straightforward and cost-effective. Fibers are produced by forcing a polymer melt or solution through a spinneret in the presence of a high electric field. This approach becomes intriguingly powerful when remarkable morphological features are combined with unique chemical, physical, or mechanical functionalisation by adding desired components with ease and control. Our current research focuses on exploring new possibilities to fabricate three-dimensional nano-biointerfaces that synergise the nanostructural induction and the bioelectrical/biochemical signaling to affect cellular behaviours, for biomedical applications in neural, bone, muscle tissue engineering and cancer modeling[1-5].

Methods
Two platforms including wet electrospinning (WES) and melt electrowriting (MEW) will be used for biofabricating 3D nanostructured biomaterials, with exterior and interior functionalization for neuromodulation, cardaic pacing and bioactive delivery synergized neuron regeneration.

Results
Drastic nanomorphology changes can be tuned on polycaprolactone (PCL) electrospun fibers using WES using phase separation during electrospinning process. Further spin coating or physical vapor deposition of 50nm of Au rendered the MEW PCL scaffold a transparent, flexible bioelectronics which could pace cardiomyocytes and enhance neuron dendrite network formation wirelessly via electromagnetic induction. Coaxial electrospinning could further load nerve growth factor and gC3N4/rGO heterojunction collectively allow optoelectric and biochemical dual stimulation for neuron tissue engineering.

Conclusions
Taken together, these nanostructured electrospun fibers offer a versatile tool for addressing a range of complex biomedical challenges through biochemical, biophysical and bioelectric stimulations across a broad spectrum of scales.

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Topic: Bioprinting & Biofabrication
Subtopic: Electrospinning, nanofiber spinning
Type: Oral presentation

TERMIS25_1295 - Development and characterization of electrospun PLGA/PCL membranes as substrates for expandable in vitro skin models

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Introduction/Objectives
Skin expansion is a surgical procedure that uses a tissue expander to stretch the skin gradually, creating more tissue for coverage in areas requiring grafts or reconstruction. The concepts of 4D printing and shape-morphing have the potential to lead to novel tissue expander designs; however, in vitro platforms are needed to evaluate skin expansion as an ethical alternative to animal models. This study aims to fabricate and characterize electrospun poly(lactic-co-glycolic acid) and poly(epsilon-caprolactone) (PLGA/PCL) membranes as expandable substrates for growing skin cells to create an in vitro skin mimetic.

Methods
Membranes were produced using electrospinning of 10 wt% or 20 wt% of PLGA/PCL in a 70:30 ratio with 1,1,1,3,3,3-Hexafluoro-2-propanol as the solvent. Commercially available BioPore membranes were used as controls. Scanning electron microscopy (SEM) was used to evaluate the morphology of the electrospun membranes, with image analysis used to determine the porosity and fiber thickness. Mechanical testing was performed to assess the membranes' Young's modulus, tensile strength, and elongation at break. Burst pressure was measured using a custom 3D printed setup and Fluigent flow controller. A custom setup was also designed to follow the expansion of 4D-printed devices held below the membrane. Two types of surface coatings were applied to improve cell adhesion: polydopamine (pDA) and type I collagen. The attachment, morphology, viability, and proliferation of dermal fibroblasts (HDFn) and keratinocytes (HEKn) on the membranes was assessed, using metabolic activity (PrestoBlue) assays as well as staining with CellTracker Green (for observing live cells) and phalloidin and DAPI (for observing fixed cells).

Results
The electrospin PLGA/PCL membranes with 10 wt% polymer concentration exhibited higher porosity compared to those with 20 wt% concentration; however, the pore size of the 10 wt% membranes was smaller, which correlated with improved tensile strength. These 10 wt% membranes demonstrated superior mechanical performance compared to both the commercial BioPore membranes and the 20 wt% PLGA/PCLmembranes. During the burst pressure tests, it was observed that both the 10 wt% and 20 wt% PLGA/PCL membranes exhibited greater resistance compared to the BioPore membranes. The mechanical properties of the membranes were suitable to allow 4D-printed expansion devices to stretch the membrane without rupturing it. Preliminary biological characterization tests showed that coating the PLGA/PCL membranes with type I collagen improved cell adhesion, but cell spreading was rather limited. Coating the PLGA/PCL membranes with pDA improved cell attachment, leading to cell spreading and proliferation; however, the pDA-treated membranes were more brittle.

Conclusions
PLGA/PCL electrospun membranes were successfully developed for in vitro dynamic skin expansion. The 10 wt% formulation outperformed the commercial BioPore membrane in terms of mechanical properties and plastic deformation, which is essential for skin expansion. Further, coating the membranes with pDA improved cell adhesion and proliferation. These membranes in combination with HDFn and HEKn provide an in vitro platform for testing of novel 4D-printed skin expansion devices.

Acknowledgement
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Topic: Biomaterials
Subtopic: Fiber-based biomaterials
Type: Oral presentation

TERMIS25_1561 - Durable immunomodulatory nanofiber niche for the functional remodeling of cardiovascular tissue

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Introduction/Objectives
Functional remodeling and prolonged anti-inflammatory responses are both vital for repairing damage in the cardiovascular system. Although these aspects have each been studied extensively alone, attempts to fabricate scaffolds that combine these effects have seen limited success.

Methods
In this study, we synthesized salvianic acid A (SA, danshensu) blocked biodegradable polyurethane (PCHU-D) and enclosed it within electrospun nanofibers to synthesize a durable immunomodulatory nanofiber niche (DINN), which provided sustained SA release during inflammation. Given its excellent processability, mechanical properties, and shape memory function, we developed two variants of the DINN as vascular scaffolds and heart patches. Both these variants exhibited outstanding therapeutic effects in in vivo experiments.

Results
The DINN was expertly designed such that it gradually decomposes along with SA release, substantially facilitating cellular infiltration and tissue remodeling. Therefore, the DINN effectively inhibited the migration and chemotaxis of inflammatory cells, while also increasing the expression of angiogenic genes. As a result, it promoted the recovery of myocardial function after myocardial infarction and induced rapid reendothelialization following arterial orthotopic transplantation repair.

Conclusions
These excellent characteristics indicate that the DINN holds great potential as a multifunctional agent for repairing cardiovascular tissue.

Topic: Tissue Engineering
Subtopic: Organoids
Type: Oral presentation

TERMIS25_230 - Unraveling endometriosis pathobiology: organoid-based models to uncover inflammatory mechanisms and related therapeutic targets

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Objectives
Although endometriosis is a prevalent and burdening gynecological disorder, it is still poorly understood. The disease's essence is the growth of endometrial tissue (i.e. inner lining of the uterus) at other places (ectopic) in the body. Current hormonal treatments are burdened with side effects, pregnancy incompatibility, and provide no cure. Our group established organoid models of the ectopic lesions (ECT-O), accurately reproducing the endometriotic tissue epithelium. These organoids can be applied to gain mechanistic insight into the disease's pathobiology and to uncover (new) drug targets. Here, we specifically focus on its inflammatory

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nature. By exposing ECT-O to cytokines and co-culturing them with macrophages (the most prominent immune cell type in the lesions and their microenvironment), we aim to mimic the inflammatory phenotype of endometriotic lesions and ultimately identify key inflammatory signaling pathways, paving the way toward novel therapeutic options.

Methods
Bioinformatic analysis on single-cell (sc) transcriptomic datasets of primary ectopic lesions, of lesion-matched derived ECT-O and of healthy endometrium tissue was applied to decipher the inflammatory profile of the respective epithelial compartments (e.g. through Gene Ontology (pathway) analysis and potential cell-cell interactions with CellPhoneDB). To recreate the inflammatory phenotype *in vitro*, ECT-O were exposed to cytokines (found upregulated in lesions and their microenvironment), and co-cultured with patient-matched M1-like inflammatory macrophages derived from blood monocytes. Bulk RNA-sequencing analysis, RT-qPCR and organoid growth monitoring (Incucyte) were applied to define endometriosis-linked inflammatory pathways and markers in ECT-O with and without cytokine exposure. Monocytes were differentiated into M1-like macrophages which were validated with flow cytometry and RT-qPCR. Organoid-macrophage co-cultures were started to assess potential crosstalk (as projected by the CellPhoneDB analysis) using immunofluorescent staining and RT-qPCR.

Results
Sc transcriptomic analysis revealed a prominent inflammatory profile in the epithelial cell population of endometriotic lesions, but disappearing in matched derived ECT-O, likely due to the absence of *in vivo* microenvironmental stimuli. Exposing the ECT-O to cytokines, known to be upregulated in lesions and their microenvironment, reinstated the inflammatory phenotype, activating key endometriosis-related inflammatory pathways, and promoted ECT-O growth. Co-cultures of ECT-O with patient-matched M1-like macrophages were initiated and will be used to investigate potential cell-cell interactions as identified by CellPhoneDB. These 'assembloid' models will be highly valuable to explore epithelial-macrophage inflammatory mechanisms in the pathobiology of endometriosis, which may lead to (new) drug targets that can be tested using these models.

Conclusions
Our organoid-based models successfully recreate several aspects of the inflammatory nature present in endometriotic lesions. The novel models offer a valuable platform for investigating the inflammatory mechanisms associated with endometriosis pathobiology and identifying related therapeutic targets. This approach holds promise for developing non-hormonal treatments that could address the limitations of the latter therapies and provide improved care for endometriosis patients.

Topic: Submit to SYMPOSIUM
Subtopic: Emerging Bioengineering Strategies for Womens Health
Type: Oral presentation

TERMIS25_405 - A detailed study on tissue regeneration and remodeling in large-volume scaffold-guided breast reconstruction using medical-grade polycaprolactone scaffolds in a preclinical porcine model

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Introduction/Objectives
Over the last few years, around two million breast implant surgeries were performed annually worldwide. Low intra- and early postoperative complication rates and quick recovery give the false impression of implant safety. Yet, an increase in over 46% of breast implant removal surgeries over the last three years highlights the severity of long-term device-related side effects. Capsular contracture, a disease caused by a foreign body reaction and associated with bacterial implant colonization, is the main reason for revision surgery. Thus, antimicrobial-coated biodegradable breast scaffolds offer a dual approach to this conundrum by preventing bacterial adhesion to the implant and providing a pro-regenerative environment within the scaffold, which then degrades over time while being replaced by the patient's tissue. Especially the restoration of large, clinically relevant volumes exceeding 100ml depicts a challenge in translational research, and therefore, understanding the regenerative processes in preclinical scaffold-guided breast reconstruction (SGBR) is rudimental for a safe and deliberate bench-to-bedside transition. We hereby present the results of a comprehensive, early timepoint study on the safety and feasibility of 100ml breast scaffolds with and without an antimicrobial coating made from tannic acid (TA) and human serum albumin (HSA) in a porcine model.

Methods
Twenty-four additively manufactured macro- and microporous 100ml breast scaffolds made from medical-grade polycaprolactone (mPCL) were implanted into four female immunocompetent Australian minipigs underneath the mammary glands (six scaffolds per pig). Scaffolds were either coated with 1%TA/5% HSA (group 1), 10%TA/1%HSA (group 2), or non-coated (group 3, control). In vivo clinical imaging using computed tomography (CT) was performed immediately, as well as 1-, 2-, and 3 months after surgery. At the 3-months endpoint, the pigs were humanely killed, specimens were retrieved and subsequently, magnet resonance imaging (MRI), biomechanical testing, and histological and immunohistochemical analyses were conducted.

Results
Postoperatively, the pigs recovered well, and no clinical device-related complications such as surgical site infection, seroma, hematoma, wound dehiscence, or scaffold extrusion were observed. Longitudinal CT imaging depicted progressive soft tissue regeneration from the periphery towards the scaffold centre in all specimens, however, with qualitative and quantitative variability, which was also detected macroscopically, in MRI, and histologically.

Conclusions
Overall, our histological findings allowed us to gain fundamental knowledge on the spatiotemporal immune-cellular and regenerative processes in preclinical large-volume SGBR for safety and efficacy evaluation purposes to bridge the gap towards clinical translation.

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