3D Bioprinted prostate cancer models: Evaluating the effect of stiffness on cellular function

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INTRODUCTION:Prostate cancer is the most common type of cancer among men aged above 65 with a high death rate. One of the reasons behind it is the limited knowledge of cellular mechanisms that lead to prostate cancer initiation and progression. This is caused by the absence of the physiologically relevant models not only for the study of disease mechanisms but also for therapy development. This study aims to develop novel 3D cell-laden models with tunable physicochemical properties for the study of basic disease mechanisms.

METHODS:Prostate cancer cells (PC3, PNT2 and LNCaP) were encapsulated in peptide hydrogels with different stiffness (alpha1, alpha2 and alpha2*) and printed using 3D Discovery printer. The mechanical properties of the constructs with the different media used for culturing the used cell lines (RPMI-1640 and F-Ham 12) were measured. In addition, the constructs go through biological assessments; including live/dead assay and histological assessment (H&E and phenotype expression test).

RESULTS:Cells were found to be more viable in charged and soft hydrogels (alpha2 and alpha2*). On the other hand, stiffer hydrogels (alpha1) can be printed with better shape fidelity. The mechanical properties of the different hydrogels differ with the media change. Phenotype expression was done for Vimentin, N and E-Cadherin, Pan cytokeratin and HIf1. The result shows that the same protein expressed in seeded and printed constructs.

DISCUSSION & CONCLUSIONS:The optimization process conducted allowed us to determine the influence of different process parameters and determine the optimal printing conditions for the production of hydrogel-based constructs with and without encapsulated cells. The influence of material stiffness on cell viability and printing process was studied. The results show that hydrogel stiffness and shear stresses (induced by the printing process) have the highest effect on cell viability. The softer hydrogels Alpha2 and alpha2* were found to make a better environment for the cells to live in compared to alpha1. Due to the fact that alpha2 hydrogel did not give a well-defined structure, alpha2* was the chosen for model preparation and testing experiments. The evaluation of the phenotype expression on both printed and seeded cells shows that the printing process does not affect the phenotype expression of encapsulated cells

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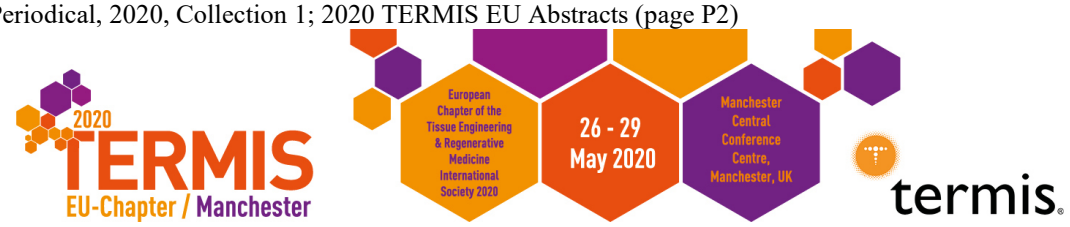
Keywords: Cancer, Hydrogels and injectable systems



Fabrication of Vascularized Engineered Tissue Scaffolds Using 3D Bioprinting Technology

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INTRODUCTION:Tissue engineering challenge lays in the ability to create large highly perfused scaffolds in which cells can grow at a high cell density and viability. Hence, the success of tissue engineering constructs is based on the presence of a capillary network with vessels located every 200 µm in all directions to prevent cell death[1]. Thus, the goal of this project is to obtain a vascularized scaffold to support the various biological functions of embedded cells. To this end, we developed a 3-step process for the creation of a perfusable vascular network: i. CAD design and printing of the network by means of a 3D bioprinter, ii. application of a hydrogel that embeds the whole network, and iii. final removal via perfusion.

METHODS:The vascular network was developed as a one floor structure with one inlet and one outlet. Vessel diameter was 2 mm and the distance between vessels was set at 200 µm. We performed computational analyses to set the perfusion velocity with respect to physiological shear stress. The vascular network was first printed in Pluronic F127 ink (CELLINK) and embedded in a hydrogel composed of 8% (w/v) alginate and 6% (w/v) gelatin (1:1). Finally, network was removed through active perfusion with deionized water at 4 °C.

RESULTS:We obtained a connected network of fluidic channel cavities within an alginate/gelatin hydrogel. After 1 hour at 4 °C the hydrogel was completely gelled. According to the computational simulations, the pump flow rate to remove the vascular network was set at 0.8 ml/min. This flow rate allows to obtain a value of velocity within physiological ranges (between 0.21 and 0.5 mm/s) and a wall shear stress largely below the safety threshold (0.5 Pa). Pluronic was successfully dissolved.

DISCUSSION & CONCLUSIONS:Building appropriate vascular structure is essential for maintaining tissue viability. Researchers have considered a variety of approaches to vascularization, however a feasible solution has yet to be found. In this project, we have demonstrated the feasibility of a new technique for fabricating a vascularized scaffold. This technique is applicable to the fabrication of a cell-laden alginate/gelatin hydrogel with embedded vascular network.

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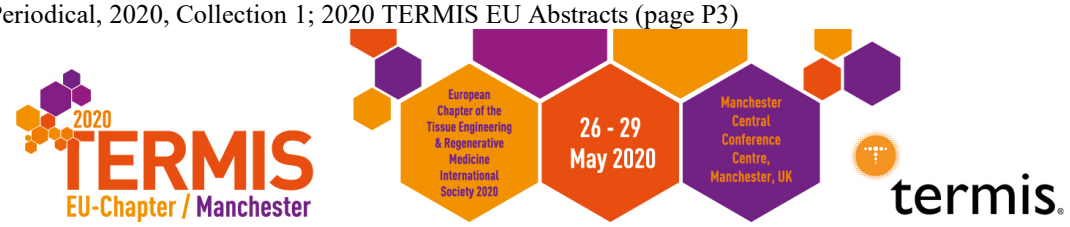
Keywords: 3D printing and bioprinting, Vascular systems / vascularisation and heart



3D laser-printed scaffolds with micro-scale features as potential candidates for growing functional neural networks

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INTRODUCTION:The functionality of the neuronal circuitry in the human brain is achieved through the formation of complex 3D networks consisting of neurons and glial cells. Research on neural networks in culture has historically used 2D platforms to study function in physiological and pathophysiological conditions. However, it is apparent that 2D culture does not adequately recapitulate the brain's 3D neuronal environment. Recent advances in the emerging field of tissue engineering and regenerative medicine have led to the successful fabrication of 3D structures that serve as scaffolds for complicated neuronal networks. However, creating models with micron/sub-micron topography that closely mimics the specificity of a human neuronal network, with the potential to be easily reproducible remains challenging. An approach that holds most promise to address the problem of creating a 3D structure with such delicate features is 3D printing, and more specifically the laser-based 3D printing technique namely Two-Photon Polymerisation (TPP). In our study, we report the examination of commercially available materials as novel candidates for neuronal scaffolds and the design of 3D scaffolds with distinct and reproducible architecture made to support a functional hiPSCS-derived neuronal network.

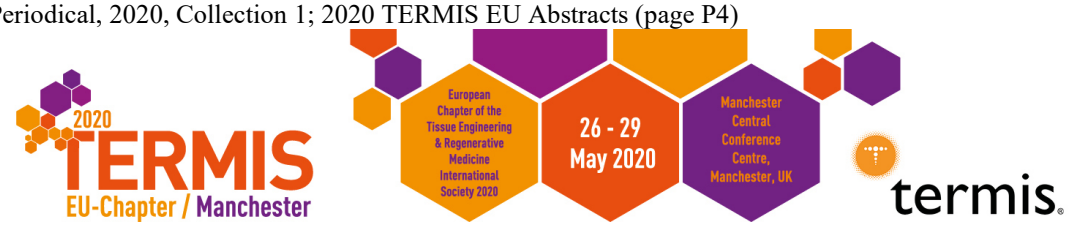
METHODS:Firstly, 3D woven scaffolds with distinct micro-scale features were designed on CAD software and then fabricated by polymerising the commercial materials LT-Clear (Formlabs) and IP-L 780 (Nanoscribe GmbH) with the Photonic Professional GT system (Nanoscribe GmbH). The 3D scaffolds were characterised for cytotoxicity and autofluorescence levels to determine their suitability for future cell culturing and calcium imaging experiments.

RESULTS:The autofluorescence outcomes showed that IP-L 780 exhibited high level of green autofluorescence, while LT-Clear did not exhibited any significant levels for either green or red autofluorescence. Moreover, post-seeding the SY5Y neuroblastoma cells we observed growth on both materials, yet more proliferation rate assessment is required.

DISCUSSION & CONCLUSIONS:Together, our results show that both materials are good candidates based on their biocompatibility and imaging potential and therefore they can be utilised for TPP micro-scaffold fabrication towards a 3D functional neuronal supporting system.

Acknowledgements:This project has received funding from the Engineering and Physical Sciences Research Council (EPSRC).

Keywords: Biomaterials, Nervous system (brain-central-peripheral / disorders)



Irradiation of pancreatic islets with 365 nm or 405 nm wavelength light

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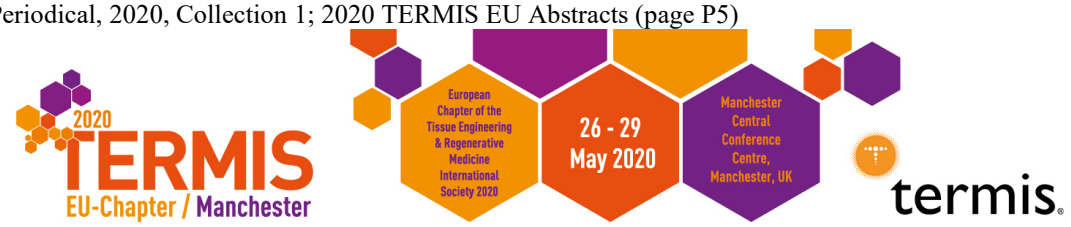
INTRODUCTION:D printing is being used more extensively in modern biomedicine. One of the problems is selecting a proper crosslinking method of bioprinted material. Amongst currently used techniques we can distinguish: physical crosslinking (e.g. Ca^{2+} and Sr^{2+}) and chemical crosslinking – the UV light crosslinking causing the biggest discussion. UV radiation is selectively absorbed by DNA, mainly in the UV-B region but also (to some extent) in UV-A and UV-C regions. DNA excitement results in typical photoproducts. The amount of strand breaks may vary depending on the period of exposition, it can also differ when cells undergo incubation after radiation. 405 nm wavelength is a novel crosslinking agent also used during bioprinting process. It is considered a safer method of stabilizing bioprinted material, with a wavelength being just above the UV light range, already in the visible light. The aim of this study was to show whether and how the time of irradiation with 405 nm and 365 nm wavelengths affect DNA damage in cell lines and micro-organs (pancreatic islets).

METHODS:The degree of DNA damage caused by different wavelengths of radiation (405 nm and 365 nm) was evaluated by a comet assay. The test was performed on fibroblasts, alpha cells, beta cells and porcine pancreatic islets after 24 hours incubation period. Samples without radiation treatment were selected as a control group. Results analysis consisted of determining the percent of cells with damaged DNA and the tail intensity evaluation. Additionally, preliminary comet assay implementing T4 endonuclease V enzyme was performed on fibroblasts.

RESULTS:The degree of DNA damage in pancreatic islets after exposure to 405 nm wavelength oscillated between 2% and 9.5% depending on the tested time period (10 – 300 seconds). However, treating islets using 365 nm wavelength resulted in damage up to 50%. The difference was statistically significant. Similar results were obtained for the tested cell lines. After adding T4 endonuclease V to the comet assay we observed a 1.5 fold increase in the amount of fibroblasts with DNA damage detected (radiation with 365nm and 405nm wavelength).

DISCUSSION & CONCLUSIONS:Crosslinking with 405 nm is better for pancreatic islets, fibroblasts, alpha and beta cells than crosslinking with 365 nm UV light.

Keywords: Other,



Development of an innovative 3D bioprintable diaphragmatic ECM-derived bioink for congenital diaphragmatic hernia repair

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INTRODUCTION:Congenital diaphragmatic hernia (CDH) is a paediatric malformation of the developing diaphragm. Current treatments with synthetic patches to repair large CDH defects lead to important side effects. Our group has recently shown that the use of biological patches obtained from the decellularization of the diaphragmatic muscle, improves the treatment of CDH in an in vivo mouse model. Unfortunately, this classical tissue engineering approach is slow, dependent on organ donation and cannot be used for large-scale production. To overcome these issues, we focused our efforts on the 3D bioprinting of diaphragmatic constructs starting from porcine decellularized extracellular matrix (dECM) components as bioink

METHODS:We explanted and decellularized pig diaphragms with 4 DET cycles by diffusion. We validated decellularization process measuring the amount of DNA and genetic material and the preservation of ECM proteins, through immunofluorescences and untargeted proteomic analyses, and evaluated the morphological characteristics by SEM analysis. We set up a gelation protocol starting from acid digestion of dECM powered, followed by temperature-induced gelation of the neutralized dECM. We performed viscoelastic, permeability, morphological and biocompatibility assays to characterize hydrogels using different dECM concentrations

RESULTS:We successfully obtained a tissue specific decellularized diaphragmatic ECM as confirmed by the decreased amount of total DNA and genetic material, preserving important ECM proteins as Collagen IV and Laminin together with unaltered muscle fibre structure and tissue organization. Proteomic analysis demonstrated that decellularized diaphragm samples were enriched with muscle specific and ECM related proteins. Rheometric analysis showed that the hydrogel temperature-induced gelation at 37°C produced an increment in viscosity in respect to a pre-gel solution at 25°C and that the hydrogels possessed a shear-thinning behaviour at increasing shear rate. Ultrastructural analysis highlighted that temperature-induced gelation leads to the formation of a compact network of fibrils into the hydrogels, and that hydrogels porosity and perfusion properties were dependent on the initial concentration of dECM powder. Finally, enzymatic degradation assay demonstrated that hydrogels were progressively degraded by collagenase II activity, confirming the biocompatibility of this material DISCUSSION & CONCLUSIONS:We developed and characterized a tissue-specific diaphragmatic decellularized ECM as a bio-compatible starting material to produce a specific bioink for the 3D printing of diaphragmatic constructs with potential clinical application in skeletal muscle malformations. This innovative approach is aimed at producing batch-to-batch identical scaffolds that will be repopulated with the cells obtained directly from each single patient, pursuing the goal of an advanced personalized regenerative medicine in a fast, automated and on demand way

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Decellularised matrices

Structural micro-heterogeneities and local viscoelastic properties of pre-crosslinked alginate gels as bioink for 3D bioprinting

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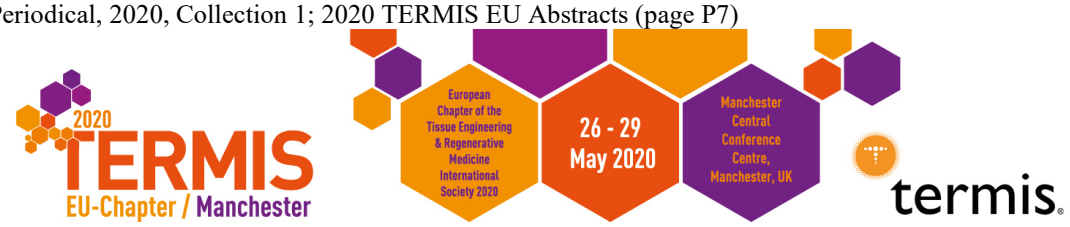
INTRODUCTION:In the last decade, interest in the field of 3D bioprinting has increased enormously. This technology strongly relies on the rheological properties of the used bioink, usually composed of pre-crosslinked biocompatible hydrogels. An accurate characterization of the ink's local viscoelastic properties and structural heterogeneities is of high importance in order to understand their role and impact on printing quality and cell viability.

METHODS:In this study, we have used classical rotational rheology and a microrheology method, namely Multiple Particle Tracking (MPT), to get new insight into microstructural and micromechanical properties of alginate gels pre-crosslinked with different divalent ions, namely $CaCl_2$, $CaSO_4$ and $CaCO_3$, covering a wide range of gelation kinetics. The gels have been investigated in water and phosphate buffered saline (PBS).

RESULTS:MPT measurements performed on pure alginate solutions show a homogeneous microstructure independent of the solvent used. In contrast, for pre-crosslinked alginate gels, measurements reveal a heterogeneous structure on the µm-scale with formation of viscous inclusions within a highly elastic matrix in both water and PBS. Viscosity, size and spatial distribution of these inclusions have been characterized, too. Additionally, these heterogeneities can be tuned by modulating the hydrogel composition, namely by changing the crosslinker and/or solvent. Heterogeneities decrease according to the following sequence of crosslinker used: $CaCl_2$, $CaSO_4$, $CaCO_3$. At given alginate and crosslinker concentrations, gels with the higher degree of heterogeneity exhibit a higher bulk elasticity as determined from rotational rheometry.
Finally, hydrogels have been printed and a correlation between microheterogeneities, cell viability and printing quality has been established.

DISCUSSION & CONCLUSIONS:These results will help to better understand the relationship between the bioink´s microstructure before printing and cell viability after printing, as well as feature size and shape fidelity of 3D printed objects.

Keywords: Hydrogels and injectable systems,



Development and characterisation of peptide-based hydrogels for 3D bioprinting applications

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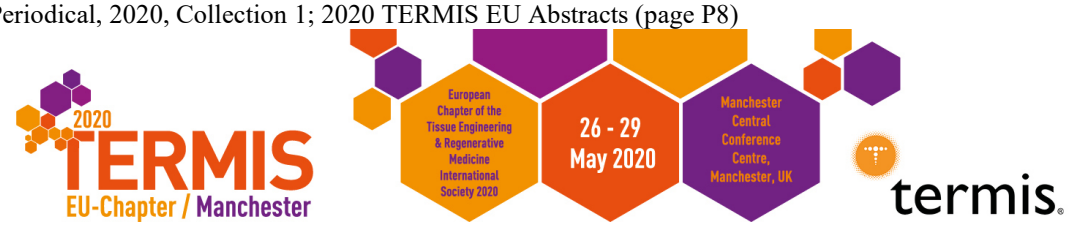
INTRODUCTION:With a growing demand for highly effective therapies, more sophisticated tissue-engineered in vitro models are required for a better understanding of the fundamental biological processes that underlie regeneration. To tackle this need and further comprehend these processes, new technologies are emerging in the tissue engineering field. The state-of-the-art technology of 3D bioprinting has the scope to achieve well-defined biological structures by printing cell-embedded hydrogels (bioinks). One of the main challenges in obtaining fully functional constructs is the lack of optimal bioinks, which not only require good printability but also a cell-friendly extracellular matrix (ECM)-like microenvironment. Peptide-based hydrogels are nature occurring, provide tailored mechanical properties and therefore, have shown significant potential as scaffold for 3D cell culture and tissue engineering applications.

METHODS:With the lack of complex tissue-engineered biological constructs and suitable biomaterials for 3D bioprinting applications, this research aims to design self-assembling peptide-based hydrogels to develop towards advanced peptide-based bioinks and novel extrusion methods for bioprinting. To characterise hydrogel printability, shape fidelity tests and rheological assessment were carried out using a BioX (Cellink) extrusion-based bioprinter, light microscopy and oscillatory rheology.

RESULTS:Printability was assessed for peptide-based hydrogels (Peptigel® hydrogels, Manchester BioGel) with different peptide sequences. Tested Peptigel® hydrogels were, alpha 1, alpha 2, alpha 4, gamma 1 and alpha X3. For this purpose, the BioX printed one-layer, 20 mm x 20 mm hydrogel grids. We first characterised shape fidelity, reproducibility and integrity for these printed constructs. Based on our tests, alpha 1, alpha 2 and alpha 4 hydrogels resulted more efficient in enhancing 3D bioprinting purposes. We further characterised printability of these hydrogels with rheological assessment. In this analysis, we observed how the tested Peptigel® hydrogels present tailored mechanical properties, viscosity and pressure for extrusion methods of 3D Bioprinting.

DISCUSSION & CONCLUSIONS:Thus far, we have successfully developed and tested peptide-based hydrogels, and initial studies indicate that these are suitable for 3D bioprinting. Next steps aim to investigate the potential of these hydrogels as bioinks. We aim to assess cell viability over a 7-day culture period of bioprinted embedded-human mesenchymal stem cells (hMSC) hydrogels, to investigate if bone differentiation could be induced, to determine how capable these constructs are to differentiate into physiologic bone phenotype.

Keywords: Additive manufacturing, Biomaterials



Bioinspired-hydrogel composites as 3D printable ink formulations

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INTRODUCTION:The 3D-printing technology holds a great potential in fabrication of scaffolds for tissue engineering with high structural complexity and in providing patient-specific designs. This study aims to develop an overarching framework for 3D-printable cellulosic materials, which can accustom application-specific properties. Ink formulations for were assessed herein by taking advantage of the fast crosslinking potential of pectin and the remarkable shear-thinning properties of nanocellulose.

METHODS:Prior to 3D-printing, the inks were evaluated by rheological and injectability tests. The reliance of the printing parameters on the ink composition was established through printing tests, while the performance of the 3D-structures was evaluated post-crosslinking in terms of mechanical properties and rehydration behavior. MicroCT was also used to evaluate the morphology of the 3D-printed objects regarding the effect of pectin/nanocellulose ratio on the geometrical features of scaffolds. Human stem cells (hASCs) were used to perform preliminary in vitro biological tests.

RESULTS:The inks were comprehensively studied regarding the influence of their properties on printing parameters. The ease of injection of gels revealed a good correlation with the precursors' composition and gave a significant insight on their printability. The rheological investigation revealed that the amount of pectin influences the rheological behavior of the suspension, preserving the shear-thinning property while increasing the yield stress, though improving the printability of ink formulations. In optimized processing conditions for all the formulated inks, a good printability of the designed 3D-pattern was attained.

DISCUSSION & CONCLUSIONS:In this research, novel bio-based inks were designed as suitable for 3D-printing by using pectin and nanocellulose. Through the rheological and printing behavior along with fast gelation properties, the bio-based inks prove to be candidate biomaterials for extrusion based 3D-printing. The formulated inks provide highly porous 3D-networks that exhibit a good structural and functional resemblance with native (living) tissue. All materials have shown to be biocompatible, and yet the best cellular response was obtained using hydrogel containing the highest amount of pectin. The predominance of the hydrocolloid within the hydrogel matrix generate a significant better cellular response and therefore compulsory properties sought in regenerative medicine might be reached.

Acknowledgements:The work has been funded by the Operational Programme Human Capital of the Ministry of European Funds through the Financial Agreement 51668/09.07.2019, SMIS code 124705. Executive Agency for Higher Education and Research Funding (UEFISCDI) and National Research Council (CNCS) are gratefully acknowledged for the financial support through the PN III research project INTELMAT no. 39PCCDI/2018.

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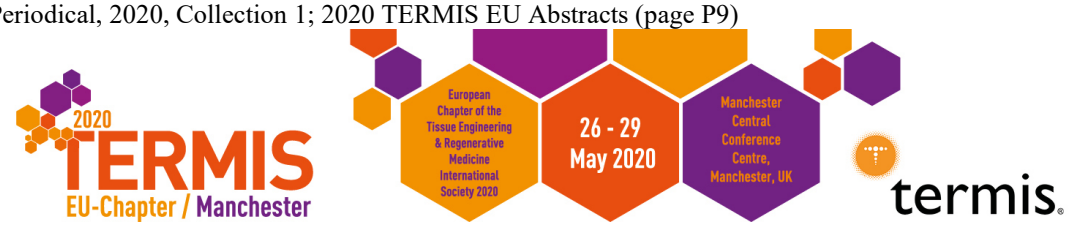
Keywords: 3D printing and bioprinting, Biomaterials



3D Printing/Bioprinting of Miniaturized Tissues Embedded in Self-Assembled Nanoparticle-based Fibrillar Platforms

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INTRODUCTION:There is a rising interest in creating miniaturized platforms through tissue engineering approaches that enable modelling of human physiology and disease. To date, several tissue models have been designed, especially based on microfluidic technology, as tissue/organ analogues, which bridges the gap between in vitro and in vivo models, offering new approaches to research in biology, medicine and pharmacology. The most common method for the fabrication of these microfluidic-based devices is soft lithography, using physiologically inert polymers like polydimethylsiloxane (PDMS). Despite various advantages of PDMS-based devices, such as optical transparency and flexibility, they require several fabrication steps and typically lack potential to emulate native cell-cell and cell-matrix crosstalk. Although numerous strategies have been explored to address these limitations, none has yet demonstrated to be simultaneously effective and economical due to the requirement for extensive processing.As an alternative to microfabricated PDMS devices,we present a unique and efficient methodology to create 3D bioprinted constructs embedded in a fibrillar support material with microfluidic channels,ECM biomimetic topography and biomolecule permeability.

METHODS:CNCs hydrogel were optimized for matrix-assisted 3D printing by evaluating the printability window by combining different % wt.of CNCs and concentration of crosslinking agent (Ca+2).Post printing, CNCs were further crosslinked with excess of Ca+2 to create stable constructs with 3D printed freeform structures and perfusable channels.To demonstarte the versatility of CNCs as support material for embedded 3D printing/bioprinting, various hydrogels obtained via thermal, ionic and photo-crosslinking were printed.

RESULTS:This unique platform allows the bioprinting of high resolution (≥50µm) patterns using low viscosity bioinks, which are conventionally non-printable. The further controlled self-assembly of CNC via ionic crosslinking under biocompatible conditions (Ca+2) leads to the formation of a non-degradable, robust and fibrillar matrix, supporting the embedded constructs. Moreover, using combinations of bioinks and sacrificial inks, this new platform allowed to directly print complex cell laden structures alongside perfusable microfluidic channels in a simple and rapid process while preserving the structural integrity and resolution of the prints.

DISCUSSION & CONCLUSIONS:Due to their ECM-like fibrillar nature and optical transparency, this CNC matrix are exceptional support materials for the development of organ-on-chip systems, which can promote biomimetic cell-matrix interaction and allows cell-cell crosstalk, both crucial to recapitulate relevant physiological microenvironments.Overall, due to its biomimetic, processing and cost advantages, this platform has thus the potential to be used as a generic method for the development of the next generation of advanced organs-on-chip.

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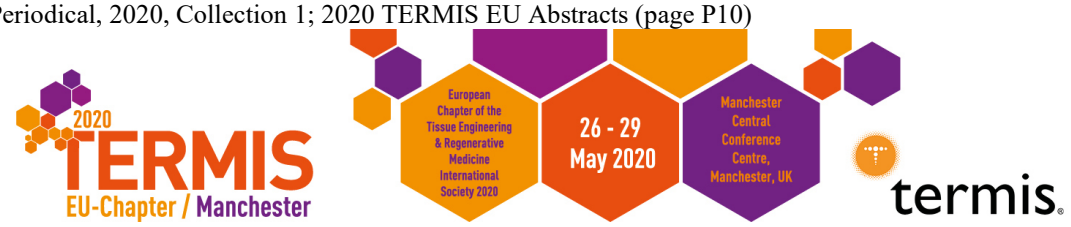
Keywords: 3D printing and bioprinting, In vitro microenvironments



Dual-functionalized visible-light responsive gelatin bioink as cartilage binding glue and matrix for 3D chondral regenereation

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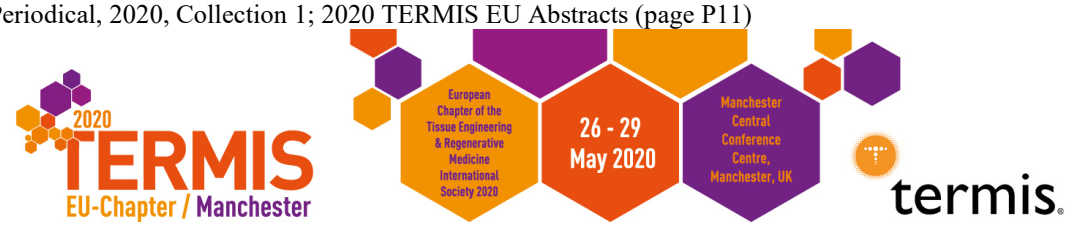
INTRODUCTION:Bioprinting with hydrogel-based bioinks hold great potential for capturing the zonal architecture of native cartilage, enhancing tissue regeneration, even via in situ printing. Yet, fixation of hydrogel constructs in cartilage is a major challenge, especially due to limited lateral integration and risks of damage to the surrounding tissue upon crosslinking. The aim of this study was to develop a printable hydrogel system with potential for in situ cartilage repair, based on a cell-laden gelatin methacryloyl hydrogel modified with tyramine moieties (gelMA-Tyr) bearing dual crosslinking capacity.

METHODS:Photo-induced gelation of both the reactive acryl- and tyramine groups was triggered in one step via visible light irradiation in presence of tris(2,2'-bipyridyl)ruthenium(II) chloride and sodium persulfate photoinitiators (Ru/SPS, λirradiation = 450 nm). The gel was assessed as matrix for cartilage tissue engineering, bioglue, and bioink for extrusion-based bioprinting. GelMA-Tyr and gelMA were loaded with articular cartilage progenitor cells and cultured in vitro (28 days). Cell viability, cartilage matrix production and mechanical properties were evaluated. Shape fidelity upon printing was tested via assessing filament stability and sagging when spanning across a series of increasingly distanced pillars and fusion of adjacent filaments due to surface tension. The tissue-binding capacity of the proposed bioink, was studied using a push-out test, with hydrogels injected (both in their sol and physical gel form) into defects cut in cartilage explants. The effect of Ru/SPS visible light crosslinking on the viability of surrounding native cartilage was compared to a common gelMA crosslinking system (Irgacure 2959, λirradiation = 365 nm).

RESULTS:GelMA-Tyr exhibited shear-thinning behaviour upon thermogelation, and could be printed using an extrusion-based platform, while showing shape retention post-printing. Both gelMA and gelMA-Tyr supported cell survival and chondrogenesis in terms of viability, glycosaminoglycans production and compressive modulus. In comparison to Irgacure, the Ru/SPS crosslinkers did not show oxygen inhibition, facilitating the use of these gels in situ. The visible-light mediated tyramine-methacryloyl dual crosslinking significantly improved the adhesive strength to native cartilage (15-fold vs gelMA crosslinked with UV). Moreover, the visible light crosslinking system resulted in higher cell viability of the native tissue surrounding the defect compared to the Irgacure/UV-A system.

DISCUSSION & CONCLUSIONS:Overall, visible light crosslinkable gelMA-Tyr hydrogels, have potential for a variety of applications in biofabrication. Thanks to the dual crosslinking mechanism triggered by Ru/SPS, this hydrogel demonstrates potential for direct delivery and integration into damaged cartilage, and is suitable bioink, further enhancing its possibilities for the repair of complex, patient-specific defects.

Keywords: 3D printing and bioprinting, Bioadhesives



Cell behavior in dense collagen hydrogels after 3D bioprinting: an experimental study

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INTRODUCTION:Extrusion-based bioprinting is a powerful tool that can be used to engineer complex tissues by producing layer-by-layer cell-laden hydrogels with controlled microscale spatial heterogeneity. Bioink is one of a key element of such biofabrication process. Recently we have developed a novel collagen bioink for direct extrusion 3D bioprinting, with high concentration of collagen [1]. However, there is no many data about cell behavior in dense collagen hydrogels. In our study, 3D cell-laden constructs with different geometrical properties were printed using Viscoll bioink, to study the cell post-printing behavior in dense collagen hydrogel.

METHODS:A highly concentrated collagen solution, 80 mg/ml (Viscoll, Imtek, Russia) was used for formulation of bioink with different collagen content. Primary human dermal cells were incorporated in the collagen solution at concentration 1x 106 cells/ ml. The syringe-based 3D bioprinter Fabion (3D Bioprinting Solutions, Russia) was used to print cell-laden collagen hydrogels. The temperature of material during the printing was set to +4°C to prevent collagen clogging in the tip. 3D bioprinted constructs were cultured during 28 days. Oscillatory shear rheology was used to study the mechanical properties variation of cell-laden collagen hydrogels during the cell culture. To study cell behavior in dense collagen hydrogels after 3D bioprinting, live/dead assay, proliferation and phenotypic marker expression determination were performed. The differences in cell survival and proliferative activity were studied depending on the stiffness of the scaffold. We also studied the dependence of the morphology and migration of dermal cells on the stiffness of the matrix using time-lapse microscopy.

RESULTS:Primary results indicated that human dermal cells retained high viability and proliferative activity in high density collagen gel. The speed of cell migration within the collagen hydrogel and the distance traveled decreased in reliable accordance with increasing concentration of collagen from 5 to 10 mg/ml, but didn't change with a further increase in collagen concentration up to 40 mg/ml. The spreading rate of cells decreased as the density of the matrix increased.

DISCUSSION & CONCLUSIONS:This result demonstrated that Viscoll bioink is a promising bioink for biofabrication and cells viability in dense collagen hydrogels after 3D bioprinting is comparable with soft collagen hydrogels.

Acknowledgements:This work was partially founded by Foundation for Assistance to Small Innovative Enterprises (FASIE), Project no. 44700. The reported study was funded by RFBR according to the research project № 19-29-04060

References:[1] https://doi.org/10.1007/s10856-019-6233-y

Keywords: 3D printing and bioprinting, Biomaterials

Pluripotent stem cells towards nephron progenitor cells: synchronization for improved differentiation efficiency

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INTRODUCTION:The application of pluripotent stem cells (PSCs) as regenerative medicine strategies represent an important for patients affected by kidney disease1. Nephron progenitor cells (NPCs), the precursors of nephrons, can be generated from PSCs. Efficient differentiation of PSCs dependent on the response of the stem cells to the external induction cues. Deriving a homogeneous population of nephron progenitor cells (NPCs), the renal precursors, is an essential prerequisite to generate a pure cell population.

METHODS:In this study, we established protocols to improve differentiation efficiency of PSCs into NPCs. The two progenitor populations, metanephric mesenchyme (MM) and ureteric bud (UB) were generated with optimized efficiency. MM and UB were combined in two-dimensions and in three-dimensions to generate kidney organoids.

RESULTS:Results showed that chemically induced reversible cell cycle arrest allows and efficient synchronization of cell that respond with high efficiency to WNT agonist CHIR99021, thus efficiently stimulating the directed differentiation of PSCs to a homogenous population of NPCs. The NPCs were characterized by the gene expression of HOXD11, CITED1, SIX2 and SALL1. The homogeneity was also validated by the expression of SIX2 and SALL1 by immunocytochemistry and flow cytometry analysis. NPCs when synchronized gave a higher yield of renal vesicles, which induced a higher number of nephrons-like structures. A hydrogel based on the ECM derived from sacrificial kidney tissue was prepared to further improve the generation of renal vesicles from NPCs. Upon induction with exogenous Activin A and FGF9 in kidney derived sacrificial ECM hydrogel, NPCs successfully formed renal vesicles. The renal vesicles were characterized by the expression of flurochrome conjugated LTL. Furthermore, ECM hydrogel was further combined with alginate-based materials and bioprinted with a microfluidics-based bioprinter showing high cell viability post-printing and up to 7 days in culture.

DISCUSSION & CONCLUSIONS:These initial steps might allow further development of renal in vitro models suitable for nephrotoxicity assays and for the development of future regenerative medicine strategies.

Acknowledgements:We acknowledge the Dutch Kidney Foundation (Nierstichting Nederland, grant 18OI17 – Innovation Call 2018) for the financial support.

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Keywords: Induced pluripotent stem cells, 3D printing and bioprinting

Optimization of the conditions of 3D bioprinting with the microextrusion method – the influence of the pressure on the viability of the cells

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Foundation of Research and Science Development

INTRODUCTION:The 3D bioprinting with the use of live cells is the newest technique from the field of biomedical engineering. One of the most important points of the procedure is saving the cells and letting them stay fully functional in the obtained bioconstruct. The most popular method used in 3D bioprinting is microextrusion[1]. Nevertheless, independently of the used bioink dosing method, it has to be kept in mind that inside the cartridge act forces that interact straightly with the cells suspended into entire bioink[2]. While using microextrusion method with the change of pressure, we change the forces acting on the cells. In this way, we may control the conditions of cells after the bioprinting process. In our work, we showed that each cell line that is used in bioprinting process should be examined in the field of the pressure influence on the viability[3].

METHODS:Cells (fibroblasts and endothelial cells) in the number of 5·105/mL are suspended in 3% of alginate and are bioprinted with the use of BioX bioprinter and with the pressure in the range of 0-200kPa. After bioprinting cells in the carrier were diluted with the use of 5mL of 1xPBS. The visualization of cells viability was performed by the FDA/Pi staining. Viability was calculated by dividing the number of live cells (green) by all counted cells (red+green).

RESULTS:The maximum pressure (post-printing viability above 80%) for human (HFF-1) and mouse (3T3-L1) fibroblasts as well as for mouse endothelial cells (BALB-5206) with the use of the nozzle 840µm for each of those 3 cell lines is above 200kPa. When we change the diameter to 200 µm the viability of the cells is above 80% when the pressure is below 190, 110 and 170kPa in the case of HFF-1, 3T3-L1 and BALB-5206, respectively.

DISCUSSION & CONCLUSIONS:We confirmed that cells viability is depended on used pressure and from the inner diameter of the nozzle used in the bioprinting process. We checked 3 cell lines and 5 inner diameters of the nozzle, and for each of the cell lines we designated the maximum pressure which may be used during bioprinting process and the cell viability won't fall below 80%.

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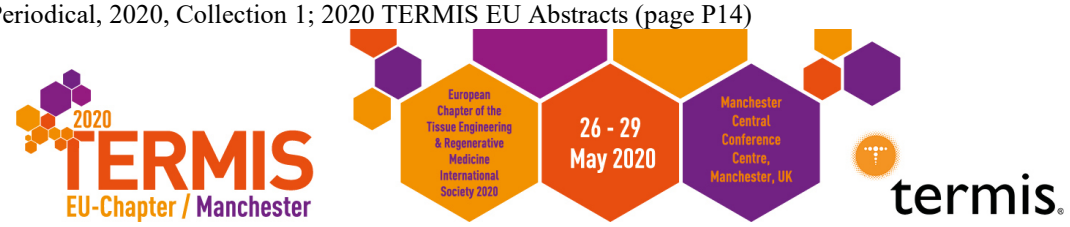
Keywords: 3D printing and bioprinting, Biomechanics / biophysical stimuli and mechanotransduction



Formation Of Artificial Blood Vessels Using ECM In 3D Bioprinting

Piotr CYWONIUK, Tomasz BRYNARSKI, Tomasz DOBRZAńSKI, Katarzyna KOSOWSKA, Marta KLAK, Grzegorz TYMICKI, Patrycja KOWALSKA, Magdalena GOMółKA, Paweł TUROWSKI, Igor ZAMORA, Andrzej BERMAN, Michał WSZOłA
Foundation of Research and Science Development

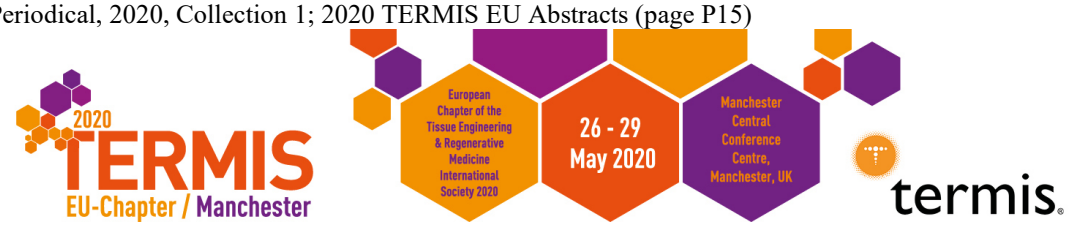
INTRODUCTION:Development of fully functional blood vessels in expanding field of artificial tissues and organs for transplantations in regenerative medicine is fundamental to provide proper perfusion and enable anastomosis with host's vascular system. The aim of this study is to establish optimal, biocompatible component which could be applied to 3D bioprinting of vessel-like tubes and promote its endothelialization.

METHODS:As protein composition of inner surface layer of vessel tube is crucial for cell attachment during endothelialization process we prepared and tested bioinks composed of extracellular matrix (ECM) isolated from several tissues differing in enrichment of types of collagen which is thought to be decisive for particular cell type adherence. To assess their applicability we 3D printed tubular structures followed by seeding with fluorescent cells commercially available as well as endothelial progenitors isolated from human blood. We also tested the most suitable approach of cell introduction via, either, cell-laden bioink or fluid injection. The effect was examined during long-term incubation in self-made perfusing chamber enabling real-time microscopic analysis and vital functions such as attachment, viability and migration were observed.

RESULTS:We generated stable vessel-like 3D structures and observed long-term cell attachment and process of endothelialization. We noticed different endothelialization rate in cells seeded onto tubes printed with particular tissue-derived ECM-based bioink.

DISCUSSION & CONCLUSIONS:Application of ECM as a component provides efficient bioink suitable for 3D printing and, simultaneously, stimulating conditions for cell maintenance and self-organization. Content of proteins naturally occurring in particular tissues differs substantially, therefore, it may affect significantly process of artificial vessels preparation.

Keywords: 3D printing and bioprinting, Vascular systems / vascularisation and heart



Production Of Detergent-free ECM-derived Bioinks For Bioprinting Of Bionic Pancreas Ready For Clinical Use

Michał WSZOŁA[1], Marta KLAK[1], Grzegorz TYMICKI[1], Andrzej BERMAN[1], Katarzyna KOSOWSKA[1], Tomasz BRYNARSKI[1], Piotr CYWONIUK[1], Magdalena GOMółKA[1], Patrycja KOWALSKA[1], Paweł TUROWSKI[1], Artur KAMIńSKI[2]
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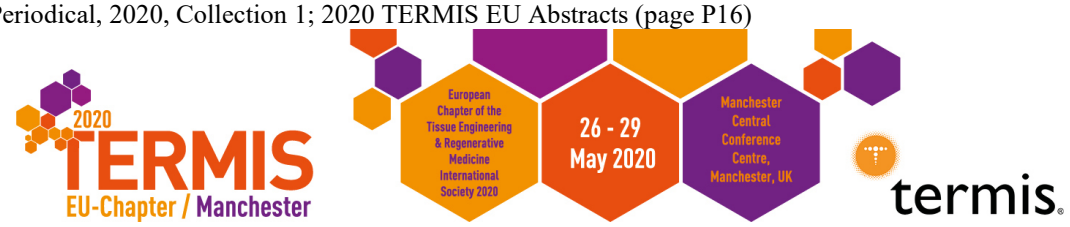
INTRODUCTION:Bioprinting is the process of creating 3D tissue analogues. For this purpose, it is necessary to produce a special bioink that will be able to recreate the natural environment for cells.Despite the progress in the process of creating biomaterial composites,no equivalent has been obtained for the complexity of the natural ECM,imitating the organ environment. Besides problems with phisico-chemical properties of bioinks for bioprinting, two major challenges are biocompatibility which influence a function of bioprinted tissue and residual detergent analyses which might be crucial in case of FDA and EMA approval for clinical use.The aim of this study was to produce detergent-free dECM-derived bioinks with biocompatibility ready for clinical use.

METHODS:The material to the decellularization was the pancreas and aorta from pigs. The procedure with the TritonX-100 detergent was used. We used standard protocol (group St) and modified with extended rising out of detergent with modified rising solution and modified temperature control(group dt-free).The bioink production protocol consists of 3 stages:(1)dissolution of dECM in a solution of with pepsin,(2)formation of dECM paste,(3)addition of cross-linking agents. Obtained bioinks were analyzed using: viscosity, and fiber morphology assessment. Residual DNA, fat and detergent concentration was assessed. Stability at normothermic conditions as well as cytotoxicity and affinity of cells to the material. Pancreatic petals were printed and in vitro tests of GSIS were analyzed on day 0,1,3 and 7.

RESULTS:The amount of detergent remaining in the group dt-free was 3.79ug/mg dECM and was significantly lower compared to the St. protocol ($p<0.008$). The fat content in the dt-free samples was almost 3.5times lower than those to the St group ($p<0.001$). The proteins structure after decellularization was not affected and the percentage of ECM proteins was the same to the control. Residual DNA for both groups was below acceptable levels. But in the new protocol the amount of DNA was further reduced almost two-fold ($p<0.027$).GSIS showed islet functionality for 7 days in printed pancreatic petals. Both control islets and those printed in alginate-based bioinks showed a maximum functionality of 24h.

DISCUSSION & CONCLUSIONS:The obtained results on bioinks have a real chance to be used in future clinical trials.

Keywords: 3D printing and bioprinting, Decellularised matrices



Biointegration And Function Of 3D Printed Bionic Pancreatic Petals On A Mouse Model

Michał WSZOŁA[1], Marta KLAK[1], Grzegorz TYMICKI[1], Andrzej BERMAN[1], Katarzyna KOSOWSKA[1], Tomasz BRYNARSKI[1], Piotr CYWONIUK[1], Magdalena GOMółKA[1], Patrycja KOWALSKA[1], Paweł TUROWSKI[1], Artur KAMIńSKI[2]

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INTRODUCTION:Despite the technologically advanced integrated systems of continuous glycemic control and insulin supply, it is still impossible to obtain results of diabetes treatment in comparison with normal pancreas function. Currently, the only way to achieve a glycemic control comparable to a healthy person in a diabetic person is by pancreas organ transplantation. The limitations of this method related to the inadequate access to organs, potential complications associated with the procedure and the loads resulting from immunosuppressive treatment mean that solutions are sought that would give a chance to be used on a large patient population and obtain glycemic control results as in healthy people. One of the solutions considered is the use of 3D printing technology using xenogenic material and stem cells. The aim of the study was to asses biointegrity and functionality of printed pancreatic petals implanted in mice.

METHODS:As part of the work, two experiments were carried out: (I) SCID mice in which type I diabetes was induced using STZ, where implanted printed constructs from biological material containing isolated porcine islets were implanted, (II) on a B6 mice into which printed constructs without pancreatic islets were implanted to assess the body's immune response. The results were analyzed using histological, immunohistochemical and biochemical tests.

RESULTS:Based on the results obtained, it was demonstrated that biochemical evaluation showed an increase in C-peptide concentration and a decrease in glucose in SCID mice with pre-induced diabetes. The implanted constructs do not elicit an immune response in B6 mice.

DISCUSSION & CONCLUSIONS:The possibility of using printed constructs as bio-integrated and functional implants has been demonstrated.

Keywords: 3D printing and bioprinting, Other

3D Bioprinting of the Media Layer of Small-Caliber Blood Vessels with a Decellularized Arterial Extracellular Matrix-Based Bioink

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INTRODUCTION:Tissue-engineered vascular grafts represent a promising alternative for the replacement of small-caliber blood vessels (SCBV). Recently, 3D bioprinting emerged as a new tissue-engineering technique capable to fabricate customized tissues on demand. One of the main challenges for the progress of this technology is the development of adequate biomaterials i.e. hydrogels capable to guide stem cell behavior, supporting cell growth and differentiation. For this purpose, the ideal hydrogel should mimic the native extracellular matrix. Thus, we evaluated the use of a vascular decellularized extracellular matrix (dECM) based bioink as a biomaterial for 3D bioprinting of the tunica media of SCBV.

METHODS:Decellularized aortic extracellular matrix-based solution was used in the present study. Upon warming to 37ºC, the solution turns into a hydrogel. Hydrogel stiffness was characterized for the gelified biomaterial. Differentiation of ASC to smooth muscle cells (SMC) on the hydrogel was induced with 10ng/mL of TGF-β1 and SM22α used as a differentiation marker. A combination of the hydrogel and 10 million cells/mL was used as bioink for 3D bioprinting of the tunica media layer of SCBV in an extrusion-based 3D bioprinter using the freeform reversible embedding of suspended hydrogels (FRESH) method. The morphology of the 3D bioprinted tissue construct was evaluated after 24 hours, 7 days, and 21 days with Masson's trichrome staining.

RESULTS:The stiffness of the hydrogel was 6.9±0.9 kPa. The hydrogel drove spontaneous (without TGF-β1) differentiation of ASC to SMC (SM22α fold change: 3.2±0.4, $p<0.0001$). The hydrogel-based bioink allowed proper bioprinting of the 3D constructs resembling the tunica media of small-caliber blood vessels. Staining of 3D bioprinted SCBV cultured for 24 hours, 7 days, and 21 days, evidenced the maturation of the construct along the time. At 24 hours, ECM hydrogel containing cells shows a porous pattern, becoming more compact the longer the construct is cultured. At 21 days, a well defined compact cell-rich vessel wall characterizes the construct. Specimens treated and not treated with TGFβ-1 did not show significant differences regarding the morphometrical parameters of the constructs. The total diameter of the SCBV reduced along time, mostly due to the compaction of the vessel walls.

DISCUSSION & CONCLUSIONS:Bioinks based on vascular decellularized extracellular matrix hydrogels offer cells a microenvironment mimetic to the native tissue, spontaneously differentiating ASC to SMC, and supporting 3D bioprinting of the tunica media layer of SCBV. This biomaterial surges as a promising alternative for the manufacturing of vascular grafts.

Keywords: Decellularised matrices, Hydrogels and injectable systems

Mechanical and biological investigation of 3D printed modular polymer-ceramic bone bricks for large bone defects

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INTRODUCTION:Bone does not have a good healing capacity for large defects. This study investigates the use of low cost, customizable, biodegradable, polymer-ceramic composite scaffold blocks for large bone defect treatment. An algorithm was developed to create blocks based on anthropometric measurements of a targeted group of patients to obtain best fitting prostheses. To control the porosity level of the blocks, a continuous tool path planning algorithm was generated. Polymer-ceramic solutions between Poly-ε-caprolactone (PCL), Tri-Calcium Phosphate (TCP), Hydroxyapatite (HA) and Bioglass 45S5 were used to manufacture the blocks. Mechanical properties were studied by carrying conducting compression tests on assembled scaffold. For biological characterization of the blocks, human adipose derived stem cells (hADSCs) were used.

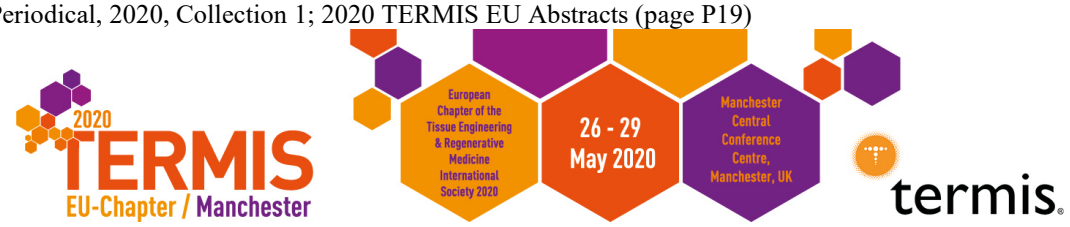
METHODS:With using the computational geometry-based algorithm, the customizable blocks were created A continuous path planning algorithm, using zig-zag and spiral like pattern in consecutive layers, is employed to create blocks with different porosity level. With custom extrusion-based 3D printer, the blocks were manufactured from PCL combined with TCP, HA, and Bioglass 45S5 in different concentrations (10wt%, 15wt%, and 20wt%). Compression tests were performed at 5 mm/min rate with 2000N load using the Instron 3344 (Instron®, USA). Human adipose derived stem cells (hADSCs) (STEMPRO, Invitrogen, USA) were used to investigate the biocompatibility of the scaffolds. Cells were cultured in MesenPRO RSTM basal media, 2% (v/v) growth supplement, 1% (v/v) glutamine and 1% (v/v) penicillin/streptomycin, (Invitrogen, USA) at incubator (37°C, 5% CO2, and 95% humidity). Cells at passage 4 were harvested and seeded on scaffolds. The cell attachment and proliferation were analysed on Day 1,7 and 14 using Alamar Blue assay.

RESULTS:Results demonstrate that the stiffness o f the scaffold decreases by increasing the pore size while it increases by increasing the material concentration. Between the different materials, PCL/TCP showed the highest results. Cells were attached and proliferated regardless of the pore size and material composition, but HA showed the highest cell activities compared with Bioglass 45S5 and TCP.

DISCUSSION & CONCLUSIONS:The mechanical evaluation study goes on to confirm that the reduction in pore size increases the compressive stiffness which was observed at most in PCL/TCP group. Biological tests with hADSC showed all the scaffolds had good cell growth and proliferation, and the highest results were observed for PCL/HA group.

ACKNOWLEDGEMENTS:This research is supported by the Engineering and Physical Sciences Research Council (EPSRC) of the UK, the Global Challenges Research Fund (GCRF), grant number EP/R015139/1

Keywords: 3D printing and bioprinting, Biofabrication



3D printing of bioactive free standing granular embedding baths

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INTRODUCTION:3D bioprinting within a granular medium has removed the barrier of printing soft materials without support[1]. The viscoelastic properties necessary to print within a granular medium can be achieved by controlling the volume fraction of the liquid phase and the granular phase. The concept of granular inks consisting of hydrogel beads has been progressively gaining attraction due to the customization potential that it offers[2–4]. In our work we present a converging point, where granular inks are 3D printed, and their resulting construct is behaving as an embedding bath that 3D printing is possible within it. The resulting architectures are spatially defined, free standing, multimaterial embedding baths.

METHODS:For the production of the hydrogel microbeads necessary for the granular inks we utilized in-air microfluidics to create 100,150 and 200 μm Alginate based beads and subsequently coated them with Poly-L-Lysine to promote cell attachment via Layer by Layer assembly. To assess the rheological properties of the granular inks and resulting constructs, parallel plate rheology was employed. The granular inks were printed in a chessboard pattern with a 21G (500μm) nozzle, and then subsequent patterns were created within them to demonstrate embedding bath capability post deposition. Smooth Muscle Cells (SMCs) and Human Umbilical Vein Endothelial Cells in 1:1 ratio were mixed with the granular ink particles in 1 million per ml cell concentration, to assess spatially defined cell adhesion.

RESULTS:We successfully demonstrate the 3D printing of granular inks that behave as an embedding bath, post deposition. Furthermore, we print within the spatially arranged embedding bath, at different timepoints, thus achieving temporal control of the construct. Moreover we tune the particle composition and the interstitial phase, to allow cell adhesion onto the particles and proliferation in the chosen compartments.

DISCUSSION & CONCLUSIONS:We have revealed that granular hydrogel inks and embedding baths can be treated as two states of one material by varying accurately the volume fraction of the liquid and granular phase. This approach enables to utilize the vast capacity of hydrogel microbead customization in the state of the art bioprinting approach of writing in the granular medium. The ability to 3D print a spatiotemporally controlled embedding bath, that instructs cells according to prior design, can be summarized in the term of a tissue blueprint.

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2:doi:10.1016/j.actbio.2019.02.054
3:doi:10.1038/s41578-019-0148-6
4:doi:10.1002/advs.201801076

Keywords: Biomaterials, Enabling technologies

Engineering 3D printed poly(lactic acid) scaffold using cerium oxide for bone tissue regeneration

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INTRODUCTION:Episodes of bone transplant is rising steeply all over the world owing to acute trauma and disorders associated with obesity and aging. In recent decades, scaffolds are used as an alternative for transplantation to mimic the extracellular matrix. The scaffold provides appropriate structural support and microenvironment for cells to attach, proliferate and differentiate to result in functional tissue. 3D printing is a rapid prototyping technique which generates patient specific complex scaffold with high porosity. Cerium oxide(ceria) has unique ability to switch between two oxidation states, i.e. +3 and +4. In biomedical applications, ceria has been used mostly in the area of soft tissues and little is reported for bone tissues. Poly lactic acid(PLA) is a non-toxic degradable polymer but lacks the bioactivity for promoting osteogenesis. The objective of present study was to fabricate and characterize 3D printed PLA scaffolds decorated with nanoceria for bone tissue regeneration.

METHODS:Fused filament fabrication(FFF) based printer was used to make 3D printed scaffolds. Circular scaffold were created in orthogonal manner to form the square pores with 500 µm diameter. After fabrication, scaffolds were hydrolyzed by sodium hydroxide (NaOH) and further conjugated with low molecular weight branched polyethyleneimine(PEI) followed by citric acid(CA) by EDC-NHS chemistry. In the final step, conjugated scaffolds were dip coated by immersing in ceria suspension prepared by oxidizing cerium chloride solution with NaOH.
Scanning electron microscopy(SEM) and energy dispersive spectroscopy(EDS) were used to characterization. Cell proliferation was studied on primary human mesenchymal stem cells (hMSCs) by using water soluble tetrazolium salt(WSTs-1) assay. Alkaline phosphatase(ALP) and alizarin red(ARS) assay were carried out in osteogenic media up to 21 days.

RESULTS:SEM analysis confirms intact structure of scaffolds after modifications steps. Pore size was found to be ≈ 414 µm, mainly because of die swelling phenomenon. Ceria coated scaffolds showed bright particles of nanometer to sub-micron size-range on the surface. PEI-CA conjugation facilitates the deposition of ceria particles on the surface. Furthermore, (EDS) confirmed the presence of cerium and oxygen. WST-1 analysis shows ceria coated scaffolds supported cell proliferation. ARS and ALP result exhibits more mineralization and ALP activity on ceria coated scaffold receptively.

DISCUSSION & CONCLUSIONS:In this study, we have fabricated porous PLA scaffold by 3D printing and functionalized with ceria. Deposition of nanoceria was confirmed. In vitro studies show that ceria coating was non-toxic to hMSCs and augment the osteogenic differentiation of hMSCs. Further physiochemical and biological characterization are currently undergoing.

Keywords: 3D printing and bioprinting, Bone and bone disorders (osteoporosis etc)

Bioprinting of Smooth Muscle Constructs for Urethral Tissue Engineering

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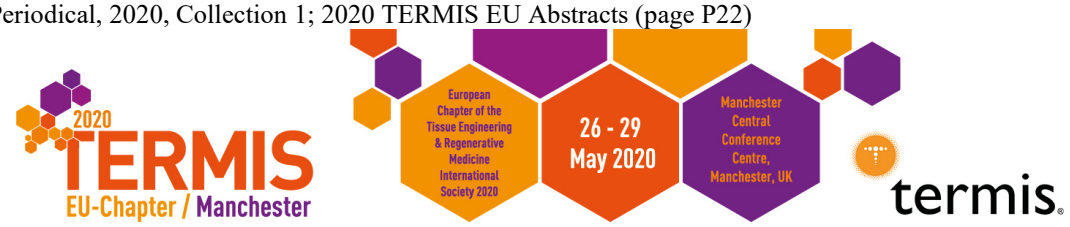
INTRODUCTION:The aim of urethral tissue-engineering is to develop biomaterial- and cell-based therapies for the repair of urethral defects. In recent years, bioprinting has emerged as a promising approach to overcome the poor cell infiltration associated with conventional scaffold-based methods. In addition, bioprinting would enable the fabrication of hierarchical cell-laden structures that mimic the architecture of native urethral tissue. One of the key challenges remains finding biomaterials with optimal printability, which could support proper assembly and maturation of cells.

METHODS:Gelatin methacrylate (GelMA, CellInk) and pure collagen (Lifeink 200, Advanced Biomatrix) were assessed in regards to their ability to support bioprinting of human urothelial smooth muscle cells (Provitro). Ring-shaped constructs (6 mm in diameter, 1 mm in height) were printed using an extrusion-based bioprinter (BioX, CellInk). Following bioprinting, live/dead and metabolic assays were used to evaluate cell viability and proliferation. Cell distribution and morphology were determined by hematoxylin and eosin staining. Upon a 10-day induction period, the maturation of the encapsulated cells was assessed by immunofluorescence staining and semi-quantitative RT-PCR.

RESULTS:Both GelMA and collagen constructs showed a high percentage of cell survival (>80%). Cells adopted a spindle-like morphology and displayed a sustained growth rate over a 5-day period. Morphological analysis revealed that cell density increased over time, mainly in the periphery of the constructs. After induction to differentiation, cells showed enhanced expression of smooth muscle actin, which was accompanied by an increased transcriptional activity of ACTA2 (α-smooth muscle actin), CALD1 (caldesmon) and CNN1 (calponin).

DISCUSSION & CONCLUSIONS:The high cell biocompatibility of GelMA and collagen-based bioinks can be explained by the presence of RGD motifs that support cellular attachment, migration and growth. Bioprinting with collagen appears more straightforward than GelMA, as it does not require an additional photo-croslinking step. However, the optimal mechanical properties of the constructs still need to be determined considering its key role in controlling the contractile cell phenotype. The results of this work, although preliminary, suggest that both GelMA and pure collagen constitute suitable bioink platforms for the fabrication of cell-laden constructs for urethral tissue engineering.

Keywords: Biofabrication, Differentiation



Cellular spheroids as raw materials for extrusion-based bioprinting

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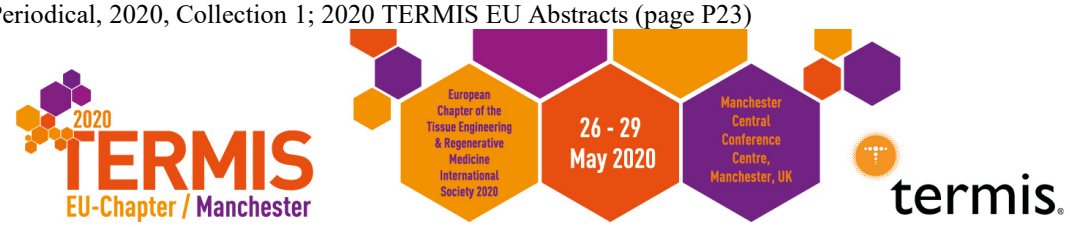
INTRODUCTION:Cellular spheroids can be used as building blocks in bioprinting technology. Bioprinting can be defined as the additive biofabrication of 3D tissues and organ constructs. This technology permits the construction of anatomically and physiologically accurate 3D biological structures and presents advantages, such as reproducibility, precision, scalability, and lower costs. Due to its qualities, bioprinting of spheroids can leverage several areas such as tissue engineering, cancer research, drug development, and disease modeling. Moreover, bioprinted tissues can be interconnected as organs-on-chips to test for drug efficacy and toxicity before the start of human clinical trials, reducing animal testing, costs and time.Therefore, the aim of this work was to explore the building-block properties of cellular spheroids by using extrusion-based bioprinting technology.

METHODS:Adipose-derived mesenchymal/stromal stem cells (ASCs) were thawed and expanded as a monolayer at a cell density of 104 cells/cm2 at 37ºC, in a humidified incubator with 5% CO2 until reach 80% of confluence. After reaching the confluence, ASCs were harvested with 0.125% trypsin and 0.78 mM ethylenediaminetetraacetic acid (Gibco, Rockville), and a concentration of 2x106 was seeded by an automated pipetting platform (Epmotion 5070, Eppendorf, Germany) onto micromolded non-adhesive hydrogels (2% agarose - Ultrapure Agarose, Invitrogen, USA - dissolved in 0.9% NaCl) with 81 circular recesses, which can generate 81 spheroids per hydrogel. Sodium alginate (2%) and calcium chloride (5%) was used as hydrogel. A commercial bioprinter was used (Regemat 3D, Spain) to dispense the spheroids in order to form atissue-engineered construct. After 7 days in culture, it was performed analysis of morphometry and viability by phase-contrast microscopy, fluorescent microscopy, and histology. The biomechanics of the tissue-engineered construct was accessed by measuring the modulus of resistance to compressive forces (Cell Scale, Canadá). RESULTS:Here we report ongoing results. It was possible to bioprinting cellular spheroids using a commercial extrusion bioprinter, without alterations in the morphology and viability of cellular spheroids. The spheroids were able to fuse, resulting in rapid post-printing building-block of the tissue-engineered construct. The tissue-engineered construct resultant showed high viability throughout the culture kinetic (< 90%), structural integrity, and higher resistance to compressive force ($p > 0.5$).

DISCUSSION & CONCLUSIONS:It was possible to biofabricate tissue-engineered constructs using cellular spheroids as raw materials. Exploring the building-block properties of cellular spheroids by using bioprinting technology enables the biofabrication of complex, anatomically and physiologically relevant tissue constructs. Hence, it has the potential to advance several areas, such as tissue engineering, drug development, and disease modeling.

Keywords: Biofabrication, Stem cells – general



The comparison of nanocellulose-alginate and nanocellulose-alginate-hyaluronic acid based bioinks for 3D-bioprinting and their suitability after different sterilization techniques

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INTRODUCTION:3D bioprinting is an emerging technology with high potential in tissue engineering since it shows effective control over scaffold fabrication and cell distribution. The fabrication of 3D bio-scaffolds requires the distribution of cells in supporting bioink in order to imitate the physiological structure of tissues. Biopolymers based bioinks offer an excellent choice for this purpose due to their biocompatibility, rheological and non-toxic properties.
Alginate (Alg) has become one of the most studied biopolymer in this field. However, the difficulties that alginate present in maintaining the scaffold structure after deposition requires the addition of high viscous components such as nanofibrillated cellulose (Nc). Moreover, the addition of extracellular matrix glycosaminoglycans such as hyaluronic acid (HA) gives the bioink the physiological similarity to achieve good cell proliferation and viability. Cell incorporation into the bioink requires sterilization assurance. Autoclave, Ultra-violet light, Gamma irradiation and Beta irradiation were widely used sterilization techniques in biomedicine fields. However, the incorporation of these techniques on 3D bioprinting procedures is still in its beginnings, and ideal sterilization treatment on bioinks is still under studying.

METHODS:In this study, nc-alg and nc-alg-HA based hydrogels were evaluated before and after different sterilization techniques. Bioinks were characterized by rheology, swelling properties, degradation kinetics, structure and morphology. Then, bioinks were passed through different sterilization techniques such as autoclave, beta irradiation and gamma irradiation, and evaluated again prior cell incorporation. Afterwards, D1-MSC-EPO cells embedded scaffolds were printed with selected sterilization technique and cell viability, proliferation and EPO secretion was analysed.

RESULTS:The experimental results demonstrated that nc-alg and nc-alg-Ha bioinks suffered physical changes after sterilization procedures. In addition, cell metabolic activity evaluation showed increasing values along the time, whereas in the cell viability assessment, the live/dead assay showed a higher percentage of live cells, all this studies indicated good cell growth and proliferation.

DISCUSSION & CONCLUSIONS:Among sterilization processes, autoclaving shown to be the best option for the sterilization of these bioinks due to their good printability. On the other hand, the addition of HA demonstrated higher biocompatibility and cell viability on the scaffolds embedded with cells.

Keywords: 3D printing and bioprinting,

MRI and NMR velocimetry mapping of fluid flow in 3D Printed porous biomaterials for Cartilage Tissue Engineering

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INTRODUCTION:Increasingly bioreactors in 3D cell culture are being used to improve cell growth and differentiation due to enhanced mass transfer and the mechanical forces they impart on the cells [1, 2]. Optimisation of tissue regeneration would benefit from methods to measure the homogeneity of flow through scaffolds, the localised forces cells experience and how these parameters change over time with the ingrowth of tissue into the scaffold. Currently outside of theoretical computed models, understanding of flow patterns in porous scaffolds and the associated mechanical stresses is limited. Optical methods often are not suitable for the opaque scaffold materials commonly used, or require the addition of tracer particles [3, 4]. Furthermore, analysis of cells post dynamic culture often involve destroying the scaffold, and do not allow for any real time measurements to be taken.

METHODS:Therefore presented here is a non-invasive NMR and MRI technique to encode velocities of flow through porous 3D printed scaffolds for tissue engineering. In this study, scaffolds were printed using Fused Deposition Modelling (FDM) with distinct pore architecture. Between scaffolds the geometry and size of pores were varied, and subjected to the same flow conditions.

RESULTS:MRI and NMR Velocity encoding successfully produced fluid flow maps for each porous architecture. Results showed that the homogeneity of flow velocity varied depending on the size and shape of pores within the scaffold.Indicating scaffold design impacts flow regimes in perfusion bioreactors.

DISCUSSION & CONCLUSIONS:As a result, it is intended the flow patterns induced by particular internal porous architecture could well influence scaffold design rationale in attempts to optimise dynamic culture methods. NMR and MRI methods could therefore be used as a technique to experimentally validate CFD models giving a more complete understanding of the interaction between scaffolds and perfusion flow.

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Keywords: Additive manufacturing, Bioreactors

Hybrid extrusion bioprinting and electrospinning setup for the fabrication of vascular grafts

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INTRODUCTION:There is a high demand for small diameter vascular grafts having mechanical and biological properties similar to that of living tissues. Tissue-engineered vascular grafts using current methods have often failed due to thrombosis, poor patency and a mismatch of mechanical properties between implanted graft and living tissues.

METHODS:We developed a hybrid bioprinting-electrospinning device that is capable of advanced biofabrication of vascular grafts. The setup can produce layered structures from electrospun fibres and cell-laden hydrogels.

RESULTS:A desktop FDM printer has been modified into a hybrid setup having one bioprinting head and two electrospinning heads. The functions of the hybrid bioprinting setup are fully controlled by Arduino controller board. The setup can reliably print tubular constructs of hydrogel around a rotating mandrel. Co-electrospinning of poly (L-lactide-co-caprolactone) PLCL and collagen fibers will be done to reinforce Methacrylated Gelatin hydrogel (also named as GelMA) for the fabrication of vascular grafts. Mechanical testing will be performed to check the burst pressure, compliance and suture retention of the bio-fabricated vascular grafts.

DISCUSSION & CONCLUSIONS:A hybrid bioprinting-electrospinning device is built to biofabricate vascular grafts with layered structure as found in natural blood vessels. This setup can make tubular constructs using hydrogels and electrospun fibers with required mechanical properties.

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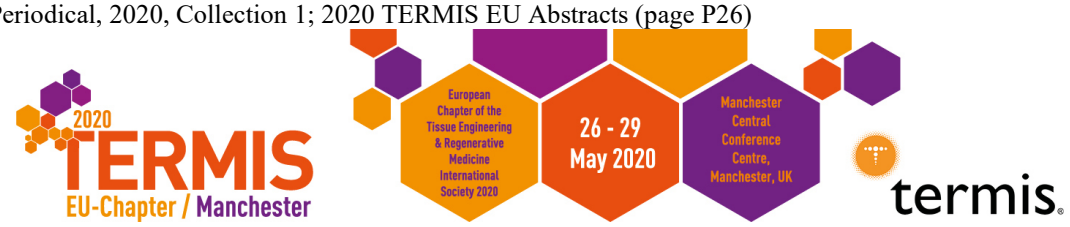
Keywords: 3D printing and bioprinting, Hydrogels and injectable systems



Laser texturing as a programming tool to elicit defined biological responses in Additive Manufacturing implants

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INTRODUCTION:Failure between implantable devices and bone bonding in the absence of infection, or aseptic loosening, remains a significant complication, affecting up to 20% of implants [1]. Such failure leads to revision surgeries with partial or total removal of the implant with higher failure rates than those for primary arthroplasty [3,4]. The increased costs and recovery times pose a heavy burden on both patient and healthcare system, critically calling for the development of novel implants with enhanced surface-bone interaction. In this regard, surface topology is known to affect the biological response of implantable devices, suggesting that careful selection of metallic implant topographies can be used to facilitate attachment and differentiation of mammalian cells. In this regard, laser ablation offers the possibility to texture implant surfaces with minimal effect on the accuracy of the final geometry.

METHODS:An array of geometrical and nature based textures to enhance the biological response of Ti64 additive manufactured implants were selected. Coupons were prepared with textures in the microscale, nanoscale and mixed patterns alongside natural textures and a polished control. The physico chemical properties of all surfaces were analysed through non contact profilometry, SEM imaging and contact angle measurements. The biological response was studied through proliferation (Live/Dead) and mineralization (Alizarin Red, Alkaline phosphatase and Polymerase chain reaction) assays of MG63 cells up to 21 days.

RESULTS:Physicochemical analysis revealed the ability of the technique to carve the desired pattern effectively modifying roughness and contact angle, known to effect surface and cell interaction. The proliferation assays revealed the ability of the patterns to modify the preferential orientation of MG63 cells after 7d of interaction. Differences were found in the mineralization assays, where grids, dual, triangles and shark skin patterns showed higher calcium deposits. These results were linked with roughness and contact angle measurements to develop a map for implant surface optimization.

DISCUSSION & CONCLUSIONS:Thus, this preliminary study demonstrated the ability of laser texturing to enhance the biological response of implantable devices, opening the door to its use in additive manufacturing technologies to tackle aseptic loosening.

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Keywords: Additive manufacturing, Biomaterials

A design approach to facilitate selective attachment of bacteria and mammalian cells to additively manufactured implants

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INTRODUCTION:Additive manufacture of patient specific implants is rapidly growing. The clinical complexity of cases that justify use of these customised devices, however typically exhibit higher infection rates compared to standardised components. Herein we demonstrate the possibility to differentially control bacterial and mammalian cell attachment on bespoke additively manufactured titanium alloy implants by optimising in situ parameters, namely the build angle. The approach offers a method to simultaneously reduce the risk of device infection while enhancing native cell attachment, which is critical to musculoskeletal implant success.

METHODS:To analyse the influence of part orientation on the surface finish of AM implants, Ti-6Al-4V cuboidal coupons were printed at sloping angles between 20 and 90° to the build plane. Physicochemical changes were studied through non contact profilometry, SEM imaging and contact angle measurements. Bacteria attachment was studied with crystal violet, SEM and Live/Dead imaging of S. epidermidis cells, while mammalian cell response was estimated with SEM and confocal imaging of MC3T3 cells. Finally, a computational model to predict surface finish and optimal orientation was developed with MATLAB accounting for melt pool radii and stacking of partially melted powder particles.

RESULTS:Roughness measurements showed a link between orientation and surface finish, increasing from 7.5 ± 0.6 µm to a maximum of 15.9 ± 1.4 µm, 20 and 90° respectively. This rise was driven by a greater number of partially melted particles on surfaces printed at higher angles, enticing a transition from hydrophilic to hydrophobic contact angles. The topographical changes resulted in a linear increase in S. epidermidis biomass, while an opposite trend was observed for cell coverage. Specifically, maximum osteoblast adhesion occurred for surfaces printed at 20° and 30°. The developed model was able to accurately predict roughness of implants which was able to minimise surface roughness and thus enhance implant performance of two implant case studies.

DISCUSSION & CONCLUSIONS:We have demonstrated the possibility of using additive manufacturing process control to selectively attach mammalian cells while hindering bacterial colonization. This novel tool has the potential to tackle the longstanding issue of infection.

Keywords: Additive manufacturing, Biomaterials

Biodegradable and Bioactive Personalized 3D Printed Implants for Maxillofacial Bone Regeneration

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INTRODUCTION:To treat maxillofacial bone atrophies, surgeons commonly use titanium meshes filled with different combinations of grafts (xenograft, autologous bone graft or synthetic grafts), in order to stimulate the regeneration of the bone defect. One of the main drawbacks of this technique is the need to perform two surgeries, one for implantation and a second one for mesh removal, increasing the possibility of side effects, pain and morbidity resulting from a surgery. Another drawback of using titanium meshes is the possibility of mesh exposure, that could affect the regeneration of the bone. The aim of this industrial project is to develop a biodegradable and bioactive patient-specific 3D printed implant as a bone substitute for maxillofacial defects regeneration, avoiding the use of titanium meshes.

METHODS:A combination of bioactive calcium phosphate-based glass microparticles (mPs) were dispersed into polycaprolactone (PCL) solution. Films of PCL with mPs were obtained and used as raw material for printing by fuse deposition. mPs dispersion and scaffolds morphology were studied by microCT and SEM images. Human mesenchymal stem cells (hMSCs) were used to asses biocompatibility and cell behavior on the scaffolds, in terms of cell metabolic activity and cell morphology by fluorescence staining (FS). Printability and personalized geometries of implants were tested.

RESULTS:Homogeneous mPs dispersion along the PCL was obtained in macro porous scaffolds with parallel pattern, observed by microCT and SEM images. A good biocompatibility was achieved according to the metabolic activity results, with a rapidly cell colonization of scaffolds observed at FS images. Other properties such as the evolution of mechanical properties and weight loss were also studied to evaluate degradation behavior. The suitability of implants geometry printed from maxillary atrophy cases, were tested with their polyamide defect models. 3D printed implants fit correctly with their corresponding bone defects models, validating implants personalization with specific geometry and their proper structural maintenance.

DISCUSSION & CONCLUSIONS:The combination of PCL with mPs used to obtain 3D printed scaffolds, could be a promising strategy to obtain biodegradable and bioactive bone substitutes for the regeneration of maxillofacial defects in tissue engineering field. This approach allows to obtain personalized scaffolds with the exact geometry of the bone defect, which also avoid the need of a second implant removal surgery.

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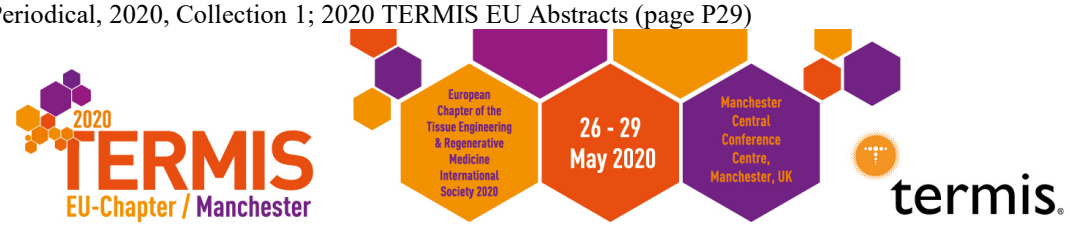
Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)



Microparticles fabricated by two-photon polymerisation with defined internal geometry for bone tissue engineering

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INTRODUCTION:Porous polymer microparticles have the potential to deliver cells and therapeutics in a minimally invasive manner. When produced by stochastic processes such as double emulsion templating it is possible to control particle size; however, the internal architecture of each microparticle is random. It has been demonstrated that pore size and geometry can be used to control mesenchymal stem cell (MSC) fate, but these architectures can only be incorporated into porous microparticles via high resolution 3D printing. Two-photon polymerisation (2PP) is an additive manufacturing technique capable of printing at sub-micron resolutions and is ideal for exploring the role of geometry on cell fate. Here, we describe a range of porous polymer microparticles fabricated by 2PP with a view to determine whether osteogenesis can be improved through architecture control.

METHODS:To rank potential photosensitive materials for 2PP a materials selection tool was developed which cross references the number of acrylate moieties on the material and MSC attachment to the material using data from high throughput polymer microarrays. Top candidates were screened and structured into a range of 100 µm diameter porous microparticles particles using 2PP (Nanoscribe Photonic Professional). Architectures were based on the Platnoic, Archimedian and Catlan polyhedra in order to give a range of well-defined pore geometries and sizes. Candidate particles were printed as arrays and MSCs cultured for three days before assessing cell interaction and attachment by by cytoskeleton staining and confocal microscopy.

RESULTS:Pentaerythritol triacrylate (PETA) and trimethylolpropane ethoxylate triacrylate (TMPETA) were identified as suitable materials due to biocompatibility and amenability to 2PP as both could readily be fabricated into a wide range of microparticle designs. Arrays of different geometry particles fabricated from the same material showed different degrees of cell interaction and ingress, demonstrating an influence of microparticle architecture.

DISCUSSION & CONCLUSIONS:Current cell studies aim to identify pore sizes and geometries which support cell interaction with and ingress into the microparticle arrays. Future work is focussed on the assessment of these materials and geometries on osteogenic differentiation. To conclude, 2PP is ideally suited to fabricating polymer microparticles with controlled internal architecture to explore the role of pore geometry on MSC fate.
ACKNOWLEDGEMENTS:We would like to acknowledge the United Kingdom Regenerative Medicine Platform 2 (UKRMP2) [MR/R015651/1] for funding this project.

Keywords: 3D printing and bioprinting, Additive manufacturing

Finite Element Modelling of Short-Stem Porous Hip Implant Design for Preventing Stress Shielding & Promoting Osseointegration

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INTRODUCTION:Currently, total hip replacement surgery is an effective treatment for osteoarthritis, where the damaged hip joint is replaced with an artificial joint. Stress shielding is a mechanical phenomenon that refers to the reduction of bone density as a result of altered stresses acting on the host bone. Current orthopaedic prostheses undergo too much stress shielding due to their solid metallic nature, which are much stiffer than the surrounding bone. During physical activities, these mechanical properties mismatch between the implant and the native bone causes the stiffer prosthesis to absorb a substantial percentage of stress, leading to stress shielding.

METHODS:This study aims to develop a hip stem which can distribute the physiological loads from the hip stem to the femoral bone. It is well known that metallic porous structures have a very good force distribution feature, these are now manufactured via metal 3D printing technology.

RESULTS:3D printed porous hip implants can help reduce this stress shielding effect and transfer a more distributed force to the surrounding bone. It has also been shown that graded density implants have an optimal lower stress value compared to the uniform porosity and conventional hip implants. This means that the graded density implants have a reduced stress shielding. Inverse Homogenization has also being used in recent studies to ensure mechanical compatibility among topology optimized microstructures This would simultaneously optimize the physical properties of the individual cells as well as those of neighbouring pairs, to ensure material connectivity and smoothly varying physical properties.

DISCUSSION & CONCLUSIONS:The architected hip implants presented in recent studies shows clinical promise in reducing bone loss while preventing implant micromotion. These graded porous structures can also promote bone tissue in growth into the implant, resulting in long term implant fixation, which eventually prolongs the life of implant and delays the need for revision surgery.

ACKNOWLEDGEMENTS:This project is financially supported by the EU via the H2020-MSCA-RISE-2016 program (grant no: 734156).
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Keywords: Additive manufacturing, Biomaterials

Anatomical investigation of the temporomandibular joint condyle for tissue engineering purposes

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INTRODUCTION:The temporomandibular joint (TMJ) is made up of the articular disc and condyle. Temporomandibular disorders affect around 10.5 million adults worldwide. Common problems in the condyle include clicking/popping, restricted mandibular range, hypermobility, osteoarthritis and rheumatoid arthritis (Morouço et al., 2016). Our laboratory is investigating novel additive manufacturing methods to tissue engineer the TMJ. However, there is limited information available in the literature to inform on the precise anatomical description of the condyle such as; cell density, extracellular matrix composition and cell type/orientation of the different zones in the condyle. We are currently investigating using histology rat and human TMJ condyles to establish their similarities and differences. This allows target clinical data to be gathered on the human tissue whilst determining the suitability of rat as an animal model to test tissue engineered TMJ condyles.

METHODS:Rats were dissected using the Fuentes et al., (2017) protocol. Tissues were fixed and stained using established protocols (O'Brien, 2017). Staining included Haematoxylin and eosin (H&E), immunohistochemistry (collagen type II) and picrosirius red (PSR).

RESULTS:H&E staining identified the four zones in the condyle. The following were found amongst the layers; fibrous layer: highlighted dense fibrous connective tissue with scattered flat cells, proliferative layer: irregular, densely packed cells with large round nuclei, chondrocytic layer: spherical cells with an increase of depth, hypertrophic layer: higher presence of chondrocytes. PSR staining highlighted a prominent presence of collagen in the condyle but did not highlight any collagen type I or III. Collagen type II was not present within the fibrous layer, but was present within the remaining three layers, with a higher presence in the proliferative and chondrocytic layers.

DISCUSSION & CONCLUSIONS:Literature states that human condyles have collagen type I and scarce type III present (Rabie et al., 2003) whereas the PSR stain used in this research showed no signs of this type of collagen, challenging previous literature (Mizoguchi et al., 2013).

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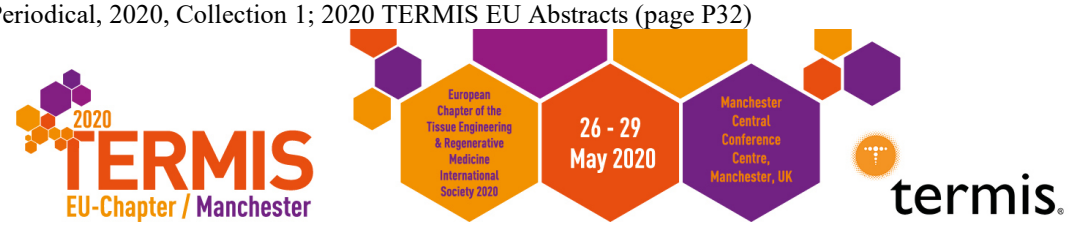
Keywords: 3D printing and bioprinting



Characterizing manufactured therapeutic chondrocytes using prognostic bioactivity markers

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INTRODUCTION:Autologous chondrocyte implantation (ACI) is a two-stage operative procedure to treat cartilage defects in which chondrocytes are isolated from an unaffected area of cartilage, expanded in vitro and then re-implanted to repair the cartilage defect. Many aspects of this process have not been optimized: in particular, monolayer expansion culture causes chondrocytes to de-differentiate and start to express general mesenchymal, or stem-like markers such as CD10, CD90, CD105, CD166, collagen-I, collagen-II and versican. Repair tissue formed from these cells has been shown to include undesirable fibrous extracellular matrix. We hypothesize that the introduction of novel 3D cell expansion techniques (bioreactors) can reduce or eliminate de-differentiation and increase the cells therapeutic potency. To facilitate this investigation and compare changes in chondrocyte therapeutic bioactivity in response to 3D culture conditions, we have developed a prognostic assay using FACS to characterize cell phenotype, enabling a rapid and high-throughput analysis of manufactured chondrocytes.

METHODS:We conducted a systematic literature review for markers that are associated with the cell types produced during chondrocyte isolation and culture, including de-differentiated and trans-differentiated mesenchymal cell types.

RESULTS:FACS markers were chosen which are present on hyaline cartilage: healthy chondrocytes, pericellular matrix and mature chondrons (CD 151, collagens II, VI, IX and aggrecan), hypertrophic chondrocytes (collagens I and X). Markers of de-differentiated cells known to appear during chondrocyte cell culture include CD90 (mesenchyme), CD9 (osteochondroprogenitor), CD146 (chondroprogenitor).

DISCUSSION & CONCLUSIONS:We have developed a FACS-based assay system to qualify, quantify and characterize the cell types and sub-populations produced during chondrocyte expansion culture. This assay is novel because it simultaneously identifies the level of de-differentiation under culture conditions and provides a ratio for immature (potentially reparative) chondrocyte/progenitor cells and the mature chondrocytes (surrounded by chondrons) which literature suggest have a role in mechano-sensing and directing in vivo tissue re-growth. This test now forms the investigative basis for high-throughput screening of cells manufactured under novel and improved bioreactor conditions, which is our current and future work.

ACKNOWLEDGEMENTS:This work is financially supported by Qatar National Research Fund (QNRF)

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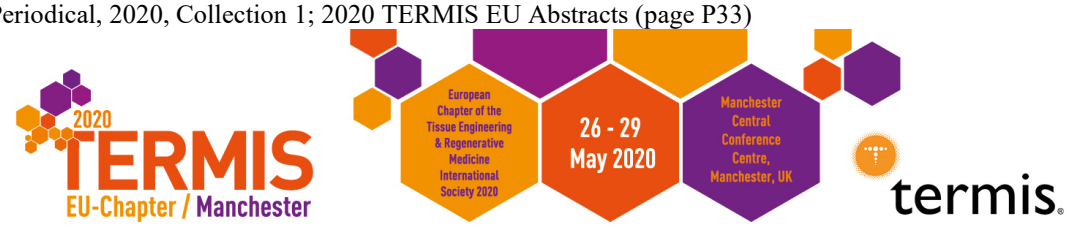
Keywords: Advanced therapy medicinal products, Cell therapy



Micronized lipoaspirate shows in vitro potential for wound healing

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INTRODUCTION:Our recent study demonstrates that micronized lipoaspirate acts as a natural scaffold for stem cells and give rise to spontaneous cell outgrowth, together with a paracrine effect on resident cells [1]. On the basis of the results obtained on cartilage regeneration, in this work we tried to demonstrate in vitro if micronized lipoaspirate can promote and accelerate wound healing overcoming the limitations of the extensive time and manipulation necessary for the use of MSCs therapy in tissue regeneration.

METHODS:Lipoaspirates were obtained from five healthy female patients undergoing a liposuction using Lipogems ® dispositive. Micronized fat was cultured 24h in RPMI with 5% FBS (0,5mL Lipogems/mL). The outgrowth study was done in a 3D collagen matrix culture using clusters of lipoaspirate transduced by Lenti-GFPF cocultured with an organ culture of healthy or mechanically damaged skin. Proliferation migration and contraction tests on fibroblasts and keratinocites have been done using MRC5 and HaCat cell lines using respectively ATP quantification kit, scratch Wound healing and collagen contraction assay. Antibacterial effect was tested using agar test and CFU count of E. coli and P. aeruginosa. Assessment of inhibition of bacterial growth was done comparing the activity of the clusters to that of hADSCs known to have an antibacterial activity [2]. To understand the paracrine effect of micronized fat, the cytokines released in culture media were assessed using the Human Cytokine Antibody Array-Membrane kit (Panomics). Moreover in order to see if lipoaspirate modify the cytokine pattern when infected, lipoaspirate clusters (0,5mL/mL RPMI) were co-incubated with 300 CFU E. coli for 24h and the cytokines released were compared to the untreated one. LL-37 peptide concentration in culture media of Lipogems® (0,5 mL) or hADMSCs (2,5×105 cells/mL) coincubated with or w/o 300 CFU E. coli for 24h was quantified using the LL-37, ELISA kit.The data were analyzed using the statistical software R (n=15; $p<0,05$

RESULTS:The ability of resident cells in microfragmented fat to grow out without enzymatic digestion and the paracrine effect on the proliferation rate, migration and contraction of fibroblasts and keratinocytes gives to Lipogems a great appeal for chronic wound repair.

DISCUSSION & CONCLUSIONS:These results together with the release of trophic/reparative cytokine and high antibacterial role of Lipogems® could constitute a promising adjuvant to antibiotic therapy, determining an effective therapeutic advantage for the healing of wounds in the field of regenerative medicine.

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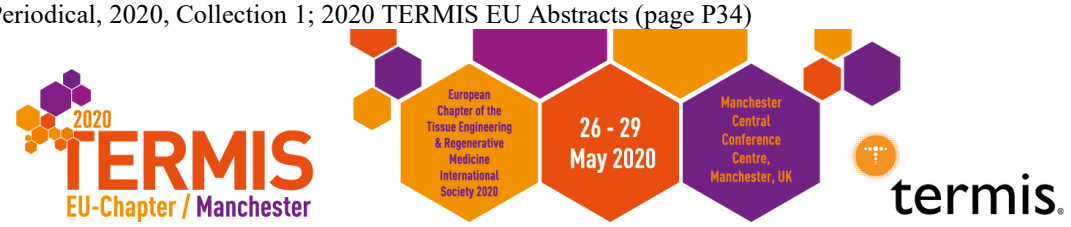
Keywords: Advanced therapy medicinal products, Wound healing



Producing biomimetic electrospun tissue engineered ligaments through the use of physiological mechanical testing

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INTRODUCTION:Currently, tensile tests are used as the main focus for assessing the performance of anterior cruciate ligament (ACL) implants; however, their suitability is not accurately assessed, due to tests not replicating physiological conditions/functioning [1]. The aim of this study is to determine the electrospun structure most comparable to the natural ligament with regards to structure and performance; hence 2D and 3D polyvinyl alcohol (PVA) electrospun scaffolds were manufactured and mechanically tested under cyclic tensile and shear loading in dry and wet conditions (not previously investigated).

METHODS:2D biomimetic electrospun scaffolds were manufactured with 12% w/v of PVA dissolved in distilled water. To fabricate 3D structures, the 2D electrospun meshes were cut in rectangles of 2 x 15 cm and manually twisted clockwise until a packed and stable filament was created to form scaffolds of: one twisted filament, three twisted filaments and three twisted/braided filaments. 2D and 3D structures were crosslinked with 25% glutaraldehyde for 24 hours. Cyclic tensile and shear tests with crosslinked and non-crosslinked samples were performed in dry and wet conditions (n=8 samples per condition). The tests were performed using an Instron H10KS, 10 cycles to 13% strain and then tested to failure, with a 100 N load cell and 5 mm/min test speed. Mean and standard error of the mean were calculated for all conditions.

RESULTS:Three twisted/braided filaments scaffolds produced a maximum tensile stress of 38.0 ± 3.0 MPa and a Young's modulus after 10 loading cycles of 148.7 ± 13.0 MPa in dry conditions, displaying properties most similar to the natural ACL [1-3]. Wet PVA scaffolds exhibited elastic behaviour with a well-defined toe region. Three twisted/braided crosslinked filament structure showed the highest maximum shear stress in dry conditions, allowing the samples to bear higher shear loads than with the other structures. The manufacturing process did not damage the nanofibres or alignment.

DISCUSSION & CONCLUSIONS:Three twisted/braided crosslinked filament structures mimicked the ACL mechanical and morphological properties, showing elastic behaviour in wet conditions and optimum tensile and shear stresses under cyclic loading, indicating significant potential for use in ACL reconstruction.

Acknowledgements:This research was funded by the Faculty of Science & Engineering, Manchester Metropolitan University.
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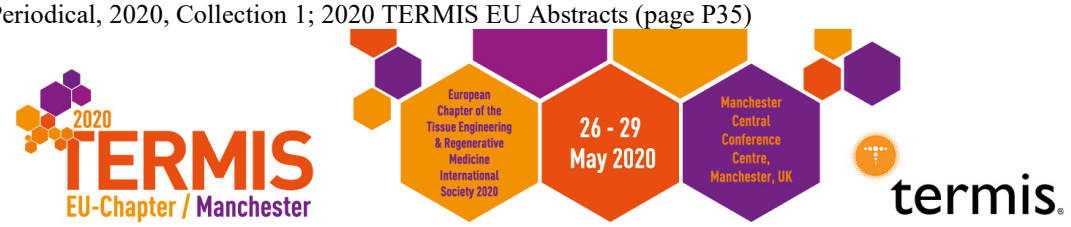
Keywords: Fibre technology, Musculoskeletal (inc ligament / tendon / muscle / etc)



Magneto-acoustic levitational biofabrication of the ring-shaped construct from tissue spheroids

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INTRODUCTION:The fabrication of human organs and tissues using three-dimensional bioprinting can be considered as a promising solution to the problem of the lack of donor organs. The key elements of technology are tissue spheroids used as building blocks for the printing of three-dimensional organ and tissue constructs [1]. The majority of current biofabrication approaches involves the use of scaffolds or labels. Recently, a new scaffold-free and label-free biofabrication method based on a magnetic levitation was implemented [2]. The combination of magnetic field with acoustic hoist of tissue spheroids can provide new features. Magneto-acoustic biofabrication of three-dimensional tissue constructs represents a novel scaffold-free and label-free.

METHODS:For levitation of tissue spheroids, the magnetic force generated by the Bitter magnet was used. To assemble of complex shape of the construct, acoustic force (acoustic radiation force) from a standing wave was used.

RESULTS:The high magnetic field with an intensity of up to 15 T was created using a Bitter magnet. To ensure levitation of tissue spheroids, a gadolinium salt in a non-toxic concentration was added to the nutrient medium. The piezoelectric transducer created standing cylindrical ultrasonic waves. In the experiments, tissue spheroids from smooth muscle cells in a paramagnetic nutrient medium were placed in a special agarose cuvette, which was transparent to the ultrasound wave and did not distort the resulting field. The acoustic radiation force formed a ring-shaped or tubular constructs from tissue spheroids. Changes in the frequency and amplitude of the wave allowed us to manipulate the size and the width of the collected construct, while the number of spheroids determined its height. Holding collected construct in the ultrasound trap for 20 hours at 37°C led to the fusion of spheroids into a solid ring-shaped living construct. The viability assessment of the construct indicated that the spheroids remained alive, and the ultrasound field had sufficiently low intensity to prevent their damage

DISCUSSION & CONCLUSIONS:Thus, in the present work we showed that a combination of magnetic and acoustic fields opens a unique opportunity for biofabrication of tissue engineering constructs with complex geometric shapes.

Acknowledgements:We acknowledge the support of the HFML, member of the European Magnetic Field Laboratory (EMFL).
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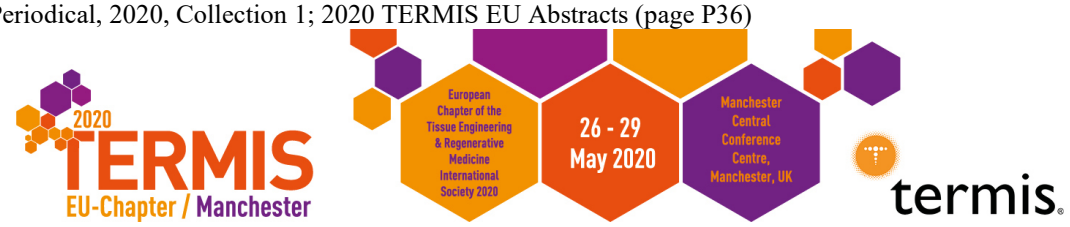
Keywords: 3D printing and bioprinting, Other



How to translate manual to additive fabrication of adipose tissue models

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INTRODUCTION:Adipose tissue plays a crucial role in the human body including heat regulation, energy and hormone homeostasis. The clinical need of adipose tissue is mainly found in reconstructive and plastic surgery, e.g. after tumor resection or to treat deep wounds. Automated fabrication techniques like boptinting allows the generation of standardized, reproducible tissue models. We focus on the optimization of the manual buildup of adipose tissue models as suspension in hydrogels or spheroids. Next, we adapt our protocols to an automated process to achieve viable, functional and physiological models for further use in biomedical sciences.

METHODS:Primary human adipose derived stem cells (ASCs) and human mature adipocytes (ACs) were isolated using skin biopsies. We improved their culture conditions, e.g. by development of several defined media as well as specific media and protocols for cocultures with endothelial cells or macrophages. 3D cuture of ASC were perfomed by cells in hydrogels or spheroid culture. Viability, differentation and function of the ASCs and ACs were evaluated by different stainings, ELISAs, quantitative assays and western blots after severeal days and weeks of culture. Moreover, we tested different biopolymers as scaffold material for 3D-adipose tissue models such as gelatin, collagen, gellan and mixed them with human ECM to provide a more physiological microenvironment.

RESULTS:We improved the culture conditions of ASCs and ACs by optimizing different media compositions and developed defined media e.g. for adipocyte culture, which supported the maintenance of the adipogenic phenotype up to 14 days. ASCs in hydrogels or spheroid 3D-culture were successfully differentiated and startd to form lipid vacuoles. This adipogenic differentiation of the ASCs and the maintenance of the ACs were analyzed by immunofluorescent staining and different assays (leptin, glycerol). Bioinks based on gelatin or gellan were evaluated and optimized by adding patient derived ECM. Cell behavior like adhesion and proliferation was improved in our ECM containing bioinks.

DISCUSSION & CONCLUSIONS:We have developed optimized culture and differentation protocols (like setup of defined media) for 2D and 3D culture ASCs and ACs as well as cocultures with other cells. For bioprinting of these models tailor-made bioinks are necessary, e.g. ECM-containing hydrogels. The ECM preparation and integration showed positive effects but should be further improved to achieve functional and stable 3D adipose tissue models by bioprinting.

ACKNOWLEDGEMENTS:Financial support was received from the Ministry of Science, Research and Art Baden-Wuerttemberg and the European Commission (#1099895).

Keywords: Biofabrication, 3D printing and bioprinting

Encapsulated nanofibrous networks within liquefied-core systems for tissue engineering applications

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INTRODUCTION:Within tissue engineering and regenerative medicine (TERM) field evolution, new tissue repair technologies have emerged. An alternative to conventional scaffolds with fixed geometries, namely hydrogels, consists of Liquefied Multilayered Capsules (LMC) [1]. Composed of a layer-by-layer membrane, a liquefied alginate core containing cells and poly(L-lactic)acid (PLLA) nanofibers, LMC allow free movement of cells across the 3D construct, while maximizing the diffusion of essential molecules. Nanofibers are of utmost interest given its function as an anchoring and assembly 3D system, mimicking the fibrous microenvironment of the extracellular matrix found in vivo. Considering their higher surface area, compared to microparticles, nanofibers may represent superior cell carrier systems. Moreover, their higher aspect ratio may aid in cell orientation. LMC encapsulating nanofibers and cells were tested in both dynamic and static conditions.

METHODS:PLLA nanofibers were synthesized through electrospinning and fragmented by aminolysis reaction. LMC were generated by electrohydrodynamic atomization technique, in which a liquid jet of alginate containing a dispersion of mesenchymal stem cells (4x106cells/mL) and PLLA fibers (1mg/mL) breaks up into droplets, by applying electrical forces. After crosslinking in calcium chloride, spherical hydrogels encapsulating cells and PLLA nanofibers were obtained. Then, layer-by-layer was repeatedly performed using poly(L-lysine), alginate, and chitosan as polyelectrolytes to produce a 12-multilayered membrane. Ultimately, the liquified core was obtained by chelation with EDTA and capsules were cultured up to 7 days in supplemented α-MEM medium. Capsules were tested using a rotary cell culture system. Static culture conditions and capsules without fibers were also tested as controls.

RESULTS:Results show the fabrication of uniform electrospun mats. After fragmentation, fibers ranged between 5 and 50µm and 1 and 2µm, in length and diameter respectively. Cells and fibers were encapsulated successfully, and Live/Dead assay showed that cells remained viable throughout the 7-day period, in both dynamic and static culture. Furthermore, actin and cell nuclei staining reveal the formation of cell agglomerates, mainly around nanofiber denser areas, in both conditions.

DISCUSSION & CONCLUSIONS:The inclusion of nanofibers promoted the formation of cell agglomerates within the LMC, containing biomimetic fibrous networks. Together with dynamic stimulus, a closer representation of in vivo conditions is accomplished, thereby possibly serving as hybrid devices implantable by minimally invasive procedures for TERM applications.

ACKNOWLEDGEMENTS:The authors acknowledge FCT for the grant BI/UI89/8662/2019, and projects CIRCUS (PTDC/BTM-MAT/31064/2017) and CICECO-Aveiro Institute of Materials (UIDB/50011/2020&UIDP/50011/2020) financed by national funds through the FCT/MCTES, and European Research Council (ERC-2014-ADG-669858-ATLAS).

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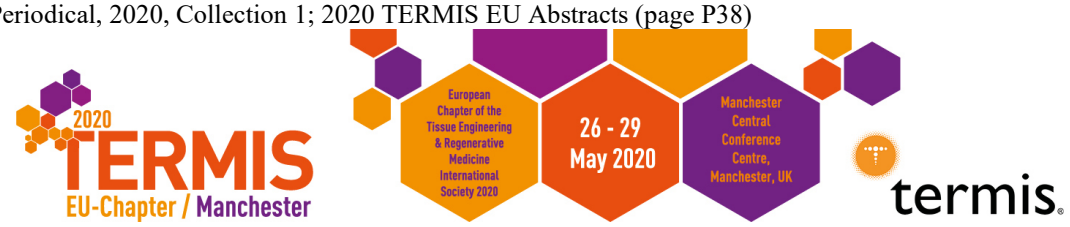
Keywords: Hydrogels and injectable systems, Polymers - natural / synthetic / responsive



3D printing of microvascular capillary scale constructs

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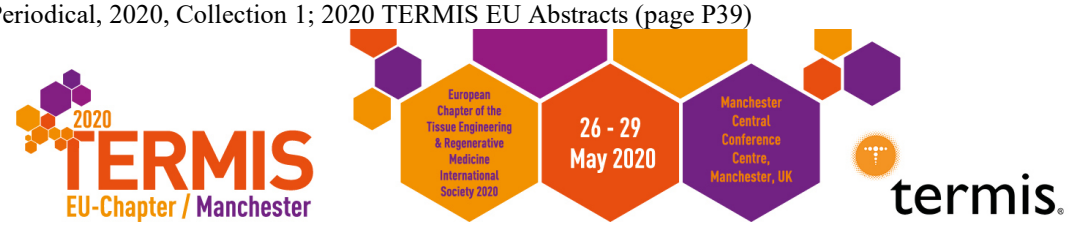
INTRODUCTION:Biofabrication of tissue constructs is often limited by the lack of a perfusable vascular network. Cells are therefore reliant on diffusion to receive its needed nutrients, limiting the size of contructs to a diffusion distance of 200µm from a capillary. We sought to develop a novel approach to the fabrication of microvascular constructs to a capillary scale by using high resolution inkjet printing.

METHODS:Pluronic F127 (PF127) was patterned with Electrohydrodynamic (EHD) inkjet printing onto a temporary mold with hierarchical geometrical features with channel feature size as small as 20µm. Gelatin methacrylate (GelMA) was synthesized using a sequential pH adjustment method as a photo-crosslinkable hydrogel matrix. This was then mixed with human dermal fibroblasts (HDFs) and casted over the fugitive PF127 ink and crosslinked with UV light at 365nm. Utilising the thermoreversible properties of both inks, the construct was then rapidly cooled to 4°C which liqueified the PF127 ink and facilitated removal. Channel patency was evaluated using FITC-dextran perfusion. The channels were then seeded with human vein endothelial cells (HUVECs). The entire construct was then cultured for 21 days.

RESULTS:Microvascular networks with complex hierarchical geometries were created by casting GelMA hydrogel over the EHD inkjet printed PF 127 fugitive templates. Channel diameter was selected at 60 µm to ensure optimal success rate. Human dermal fibroblasts (HDFs) were encapsulated within the hydrogel matrix accompanied with the post-seeded HUVECs for the endothelization of the microchannels. The microvascular tissue constructs could be maintained by perfused media over 21 days in culture, exhibiting superior cell viability over the unpatterned matrix controls (92% vs 13%). With the 3D co-culture of HDFs and HUVECs, the perfused microchannels were covered by a confluent endothelium monolayer whilst a surrounding support tissue was formed by the populated fibroblasts.

DISCUSSION & CONCLUSIONS:We show that it is feasible to print capillary sized channels using the inkjet printer and that this can potentially be a building block for upscaling to three dimensions and ultimately to dimensions required to be used in clinical practice.

Keywords: 3D printing and bioprinting



Heterogeneous tissue engineered constructs in assessment of fundamental cell behavior

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INTRODUCTION:Currently, a great number of factors, which influence the cell behavior and tissue formation at the injury site when using scaffolds are known. Those factors include the scaffold material, mechanical characteristics, surface roughness, porosity and many others. In this study, we focused on a different basic parameter affecting the cell behavior – structural heterogeneity.

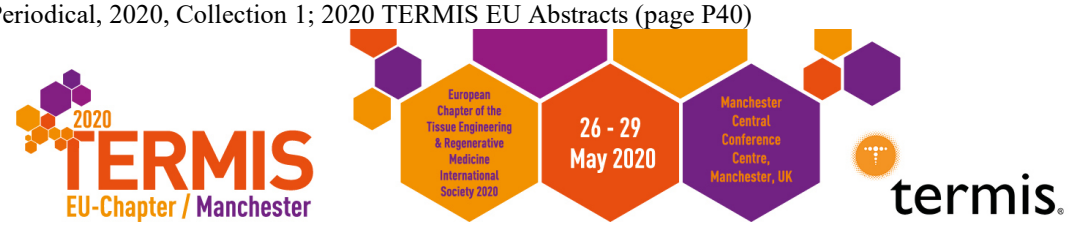
METHODS:As a model to study the formation of biological tissue in 3D microenvironment, we have developed scaffolds with a heterogeneous architectonics that mimic the structure of native vascularized tissues. The heterogeneous scaffold were obtained by two-photon polymerization technic. Similar scaffolds with a homogeneous structure were used as a control group. The scaffolds were seeded with mesenchymal stromal cells and studied by the different technologies of optical bioimaging.

RESULTS:We analyzed the cell behavior and metabolism by novel optical bioimaging technologies including multiphoton microscopy and FLIM (Fluorescence-Lifetime Imaging Microscopy). Indeed, we found that the MSC behavior and metabolism have a great differences between the heterogeneous and standard tissue constructs. The main differences relate to cell migration, proliferation, and metabolic pathways. For targeted analysis of metabolic pathways occurring in the cells fluorescence lifetimes of energy cofactors NADH and NADPH were assessed. We found that the level of protein-free and protein bound NAD(P)H for the cells on the heterogeneous and standard scaffolds was not the same. This may indicate a different contribution of glycolysis and oxidative phosphorylation to the overall cell metabolism.

DISCUSSION & CONCLUSIONS:Despite of a number of significant advances in tissue engineering, basic aspects of fundamental biological knowledge on formation of functional vascularized tissue in 3D microenvironments are still missing. Body tissues have a complex heterogeneous structure, which is often overlooked when modeling a particular cell niche or microenvironment. Since existing models of 3D-microenvironment do not take into account this most important parameter - structural and cellular heterogeneity, the research results are not quite complete. The heterogeneous scaffolds can significantly expand the fundamental knowledge of cellular behavior and the formation of various types of tissues in vitro and in vivo.

Acknowledgements:This work was supported by grant of the Russian Science Foundation № 19-75-10008.

Keywords: Biofabrication, Imaging – advanced



A Biomimetic Macroporous Hybrid Scaffold with Sustained Drug Delivery for Enhanced Bone Regeneration

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INTRODUCTION:Bone regeneration is a complicated physiological process regulated by several factors. In particular, a bone-mimicking extracellular matrix and available osteogenic growth factors such as bone morphogenetic protein (BMP) have been regarded as key factors to induce bone regeneration. In this study, we developed a bioinspired hybrid scaffold with sustained release of BMP-2 that would result in enhanced bone formation.

METHODS:This hybrid scaffold, composed of cryoelectrospun poly (ε-caprolactone) (PCL) (CE) surrounded by gelatin/heparin cryogel (GH), is designed to overcome the drawbacks of relatively weak mechanical properties of cryogel and poor biocompatibility of electrospun PCL. The GH component of the hybrid scaffold provides a hydrophilic surface to improve the biological response of the cells while the CE component increases the mechanical strength of the scaffold, to provide enhanced mechanical support for the defect area and a better environment for osteogenic differentiation.

RESULTS:After analyzing characteristics of the hybrid scaffold such as porosity, degradation rate, swelling ratio and mechanical properties, we confirmed that the hybrid scaffold group shows an enhanced cell proliferation rate compared to a CE-only scaffold.Furthermore, BMP-2 was embedded in the CE fraction to allow its sustained release for continuous osteogenesis.

DISCUSSION & CONCLUSIONS:We confirmed the sustained drug release kinetics of BMP-2 by ELISA, and we were able to demonstrate the osteogenic effect from this hybrid scaffold via in vitro.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

Functionalization of Human Palate and Oral Mucosa Substitutes for Craniofacial Tissue Engineering Applications

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INTRODUCTION:Biocompatible biomaterials have shown promising results in oral mucosa and palate tissue engineering. However, in vitro differentiation and synthesis of key components of the oral mucosa is very preliminary in these bioartificial tissues (1). Thus, novel biofabrication methods allowing the efficient generation of fully functional, mature oral mucosa substitutes are in need. In this regard, biomaterial functionalization can be achieved by introducing functional molecules, cells and other tissue components. In this work, we carried out a functionalization study of fibrin-based biomaterials using oral mucosa microexplants and we evaluated their potential usefulness in oral mucosa tissue engineering.

METHODS:Functionalized artificial oral mucosa substitutes were generated by immersing small palate human oral mucosa microexplants within a fibrin-agarose scaffold, and were kept in culture for 4 weeks. Vimentin immunohistochemistry was used to identify stromal cells in the scaffold. Synthesis of glycoproteins and proteoglycans was assessed by PAS and Alcian Blue histochemistry staining, respectively, and collagen fibres were identified by Picrosirius staining and collagen type I immunohistochemistry. Staining signal intensity was quantified from 0 (no expression) to 100% (maximum expression) using human native oral mucosa samples as positive controls.

RESULTS:Vimentin-positive cells tended to remain into the microexplants during the first 2 weeks, but migrated into the scaffold and spread after 3 weeks of culture. Expression levels of proteoglycans were closely similar to control native oral mucosa after 4 weeks, whereas glycoproteins showed 63.09% of the expression in control oral mucosa. For fibrillar proteins in functionalized tissues, collagen detected by Picrosirius showed 49.8% intensity of the native oral mucosa after 4 weeks, with 70.23% expression of collagen type I as determined by immunohistochemistry.

DISCUSSION & CONCLUSIONS:Functionalization of the scaffold using human oral mucosa explants succeeded in generating a tissue engineered palate oral mucosa with high levels of maturation and differentiation as compared to previous reports (1,2). This type of functionalization allows oral mucosa fibroblasts to migrate and spread within the scaffold, inducing the synthesis of crucial extracellular matrix components.

ACKNOWLEDGEMENTS:Supported by Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) from Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grants FIS PI18/332 and PI18/331 (co-financed by ERDF-FEDER, European Union) and by Consejería de Salud y Familias, Junta de Andalucía, Spain, grant PI-0442-2019.

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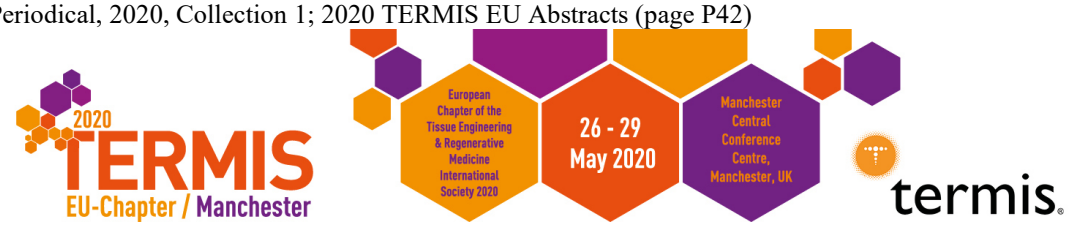
Keywords: Biomaterials,



C-MYC pathway modulation in cell activated by platelet lysate stimulation

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INTRODUCTION:Platelet Lysate (PL), containing platelet growth factors, is able to support isolation, expansion and proliferation of Mesenchymal Stem Cells derived from different tissues and cell lines as widely reported in the literature. The PL effect on different types of cells and the molecular mechanisms involved in these processes remains poorly understood. We focused our attention on a family of highly conserved proteins involved in different cellular mechanisms such as cell growth, proliferation and apoptosis, C-MYC's family. The three proteins of C-MYC gene have the same c-terminal part, differing in the N-terminal region due to an alternative translation at the start site: C-MYC1, C-MYC2 and c-MYCS differently expressed during cell growth.

METHODS:MSCs obtained from Bone Marrow, Adipose tissue Condrocyte, Osteoblast, Amnotic Stem Cells and HeLa cell line, were isolated and cultivated in medium supplemented with 10% FBS or PL 5%. These cells were compared to cells previously expanded in FBS and stimulated for different time with PL. C-MYC expression was evaluated by immunoblot analysis of cell lysates, while cell proliferation was investigated by immunofluorescence analysis for the proliferation marker ki67 and Vybrant® Apoptosis Assay Kit. The effect of methionine on C-MYC expression was evaluated in cells treated with PL.

RESULTS:Cells treated with PL showed a high proliferation rate respect to cultures treated with FBS. All cells isolated from the tissue in FBS and then stimulated at different time with PL or isolated from the tissue directly in PL, expressed the C-MYC1 isoform, which was absent when cells were cultured in the presence of FBS with a statistically significant difference. The same cells expressed the C-MYC2 isoforms independently from the culture conditions. For the cell line, the result showed a scene slightly different with a modest expression of this isoform also in the standard condition. Immunofluorescence analysis performed with a Ki67 specific antibody indicated that MSC cultures showing C-MYC1 protein expression were in a proliferative stage. C-MYC1 resulted to be expressed also from cells treated with PL in presence of methionine. The results showed that C-MYC1 protein levels in PL treated cells were unaffected by methionine.

DISCUSSION & CONCLUSIONS:All these experiments showed that PL was responsible for the induction or increasing of C-MYC1 isoform in primary cells culture and much more slightly in the cell line. These results pave the way to a deeper molecular study in order to understand peculiar differences of PL stimulation on various types of cells.

ACKNOWLEDGEMENTS:Starstem project 761214-16

Keywords: Stem cells - general, Multipotent (mesenchymal) stem cells

Interaction of mesenchymal stem cells in contact with porous titanium alloy

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INTRODUCTION:This work aims to develop a new titanium alloy with better mechanical and biological compatibility, through non-toxic elements for orthopedic purpose. These characteristics are attributed to the enrichment in β phase titanium achieved by the addition of niobium (Nb) and tin (Sn) in appropriate proportions. The biocompatibility is also related to the material surface characteristics, such as porosity and roughness. Therefore, magnesium (Mg) was added as a space holder to the Ti-34Nb-6Sn system to promote porous formation.

METHODS:For the preparation of the materials was used the powder metallurgy technique combined to the space holder. Alloys interaction was investigated when in direct and indirect contact with equine bone marrow derived mesenchymal stem cells (bmMSCs). For the indirect contact, cells were plated (1x104 cells / well) in 96-well plates and the alloys were inserted into culture medium. Both were incubated at 37ºC and 5% CO2. After the cells reached the semi confluence, the control group received conventional medium and the treated group received the conditioned medium with the material. After 24h the plates were treated with 3- [4,5-dimethylthiazole-2-yl]-2,5 diphenyltetrazolium bromide (MTT) salt to assess cell viability. The absorbance was read at a $\lambda = 570$nm. In the direct contact, bmMSCs were plated at the same conditions of indirect contact, but on the alloy surface to evaluate cell interaction. After 72 hours, the cells were evaluated by Scanning Electron Microscopy (SEM). For the statistical analysis, the nonparametric T-test was used and the significance level was 5%.

RESULTS:In 24 hours by MTT assay the control group and the treated group showed no significant difference ($P > 0.05$). Within 72 hours by SEM the cells showed good adhesion both inside and outside the pores. The Ti-34Nb-6Sn alloys made when in indirect contact to bmMSCs showed no harmful effects.

DISCUSSION & CONCLUSIONS:Possibly, to the absence of toxic elements in its constitution. When in direct contact, the bmMSCs were totally anchored on the surface with a spreading morphology and cytoplasmic projections presence. These features can help the interaction with each other, favoring the proliferation and adhesion process. The presence of Mg by the not total evaporation, it may help to cell attachment inside the pores due to its biocompatibility and thus contribute to a good fixation and osteointegration when implanted.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

Developing novel 3D hydrogel-based models of the human mammary gland to investigate breast cancer initiation

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INTRODUCTION:Women with dense breast tissue are 4-6 times more likely to develop breast cancer than women with less dense tissue. Dense breast tissue is associated with an increase in aligned fibrillar collagen bundles, which increase the stiffness of the stroma, which is directly correlated with an increased risk of breast cancer incidence in women[1].

How stiff breast matrix promotes breast cancer development is poorly understood. Currently, we understand that mammary epithelial cells (MECs) can detect and respond to environmental mechanical cues via mechanosensitive signalling pathways[2]. However, these pathways are ill-defined.
Investigating mechanosignalling events between MECs and their environment requires a mechanically tunable, well-defined and reproducible 3D model of the human mammary gland. Biomaterials currently used to model the mammary gland suffer from limitations that inhibit their ability to recapitulate the native breast environment, highlighting the need for a biomaterial that meets our requirements[3].

METHODS:PeptiGels (Manchester BIOGEL), a family of synthetic, biocompatible, self-assembling peptide hydrogels that have been recently used for several biomedical applications, were used[4–5]. Non-malignant, human MECs (MCF10a) were encapsulated in PeptiGels for three weeks and probed for markers of acinar structure development to test their compatibility for MEC culture. Modifications made to gels included diluting them with sterile water and mixing in matrix proteins.

RESULTS:Current attempts to functionalise PeptiGel Alpha4 for MEC culture have successfully altered the gel's mechanical properties and increased MCF10a viability and cluster formation. However, none of the clusters displayed markers of acinar structure formation.

DISCUSSION & CONCLUSIONS:PeptiGel Alpha4 is a synthetic peptide hydrogel that is well-defined, biocompatible and tunable. We show that PeptiGel Alpha4 can support MEC viability and is amenable to chemical and mechanical tuning. These results suggest that PeptiGel Alpha4 can be used to make an accurate model of the human mammary gland which will help us define MEC-matrix interactions.

Acknowledgements:Funding provided by Manchester BIOGEL and UKRMP.

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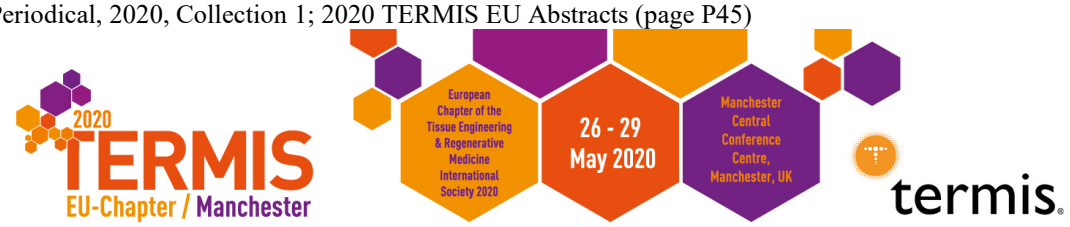
Keywords: Hydrogels and injectable systems, Cancer



The evaluation of tissue biocompatibility of tissue-engineered trachea in non-human primates

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INTRODUCTION:Multifocal and extensive tracheal stenosis is a life-threatening problem. Nowadays despite the improvement in surgery this categories of patients for whom the choice of effective surgical strategy is particularly difficult. Tissue-engineered organ represent interesting and potentially important strategies for the treatment of patients with various disorders, especially with tracheal stenosis.
The aim of the study was to evaluate the biological compatibility of the tissue-engineered trachea based on a polyethylene terephthalate (PET) scaffold and allogenic mesenchymal stem cells (MSC) in experimental non-human primates.

METHODS:The study was carried out on 6 nonhuman adult male primate Papio hamadryas at Research Institute of Medical Primatology. Laboratory animals were kept and all manipulations on them carried out in accordance with the requirements of Bioethics Committee.
The tissue-engineered trachea had composed of synthetic 3D-scaffold seeded with primate allogenic MSC.
3D-scaffold was created from PET by electrospinning and cells were obtained from the bank of Research Institute of Medical Primatology.
After 72 hours cultivation in the rotating bioreactor the samples of tissue-engineered trachea was used for subcutaneous heterotopic implantation into the scapular area of the back for local reaction. The samples existed from heterotopic position were removed after 30 days and histological examination was done.

RESULTS:The surgical intervention proper had no negative effect on animal status. The primates remained healthy and active throughout the entire period of observation; no complications were detected.
Before the implantation tissue-engineered trachea was tested for biocompatibility with MSC. Cell viability was 73.4±10.2%. Morphological analysis of tissue-engineered samples showed chaotically located synthetic filaments to which cells adhered, confirmed by DAPI staining.
The morphological evaluation of implanted samples showed a fine capsule, which enveloped the implanted scaffold; the capsule was presented by compact fibrous connective tissue. Aseptic inflammation was detected in the samples, no demarcation roll from neutrophils was found. Highly functional macrophages were mainly located around the implant and were presented by large cells with intensely basophilic cytoplasm and marked vacuoles. Immunohistochemical reaction with antibodies to vimentin detected cells of mesenchymal origin in the tissues adjacent to the scaffold and inside. Importantly, that vimentin-positive cells were also detected in the capsular vessels, which was characteristic of endothelial cells.

DISCUSSION & CONCLUSIONS:In vitro studies of tissue-engineered tracheal samples detected the scaffold biocompatibility at the cellular level. The presence of a fine connective tissue capsule without manifest fibrosis, no signs of microcirculatory disorders and immune disorders indicate the biocompatibility at the tissue level and absence of toxicity.

Keywords: Polymers - natural / synthetic / responsive, In vivo and animal models

Biodegradable Conductive Scaffolds from Poly(glycerol-sebacate) and Magnesium Alloy as Peripheral Nerve Regeneration Constructs

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INTRODUCTION:Peripheral nerve injuries are a serious clinical problem leading to demyelination of the nerve and affect the patient's quality of life. Nerve conduits have been used in the treatment of large scale (~ 4-5 mm) nerve injuries. They should offer adjustable geometry, promote cell proliferation and migration, and minimize scar formation [1]. In this study, a fully biodegradable and conductive conduit material is prepared using poly(glycerol-sebacate) (PGS) and AZ31 magnesium alloy. The final conduit is fabricated with a 500 µm conductive magnesium channels. The construct is evaluated by structural and in vitro characterization.

METHODS:Poly (glycerol-sebacate) (PGS) elastomers are synthesized using microwave irradiation and subsequent in vacuum crosslinking and analyzed by using SEM, FT-IR, DSC, degradation and tensile tests. The microchannels were then created on the elastomer surface with CO_2 laser (Nova 7, Aeon, Australia). The AZ31 magnesium alloy foil is used to provide conductivity to the conduit. The elastomer sheets were coated via thin-film deposition using sputter (PVD handy 2M, Vaksis, Turkey). The conductivity of the construct was evaluated by the Four-Point Probe method. In vitro activity of the nerve conduit was performed using nerve cell-like PC-12 and Schwann (S42) cell lines.

RESULTS:Mechanical and physical properties of PGS elastomer were optimized to the nerve tissue by changing crosslinking time and monomers concentration. FTIR and DSC results confirmed the polymerization of elastomer. SEM images indicated a channel width of 500 µm and a depth of 850 µm. The magnesium layer thickness was measured as 250 nm by stylus profilometer (p6, KLA-Tencor, USA). The percent elongation value was calculated as 95%. In vitro studies confirmed cytocompatibility and good cell adhesion and proliferation.

DISCUSSION & CONCLUSIONS:A fully degradable and conductive PGS-Mg alloy construct was prepared and proposed as a potential nerve conduit candidate. This construct has the advantages of flexibility, conductivity, and the ability to align the cells.

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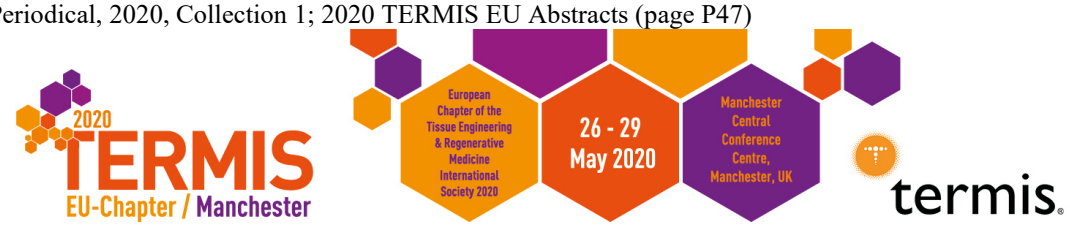
Keywords: Polymers - natural / synthetic / responsive, Nervous system (brain-central-peripheral / disorders)



Ferroelectric Scaffolds for Tissue Engineering Applications

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INTRODUCTION:A recent innovation in tissue engineering in the past decade had been the introduction of piezoelectric materials, which convert mechanical stress into electrical energy. This electricity can then be utilised to actively stimulate tissues, enhancing their natural ability to regenerate. Though the field has seen much growth, it lacks a material which is suitable for a variety of cell and tissue types. Cellulose is a compelling candidate, being biodegradable in physiological pH, bioabsorbable in vivo, highly processable, chemically functionalisable, mechanically tuneable and in recent literature has been observed to have a porosity which can be templated by freeze casting. This versatility allows a two-fold approach to its use as a scaffold.

METHODS:Using a novel combination of freeze-casting and cold-regeneration of cellulose we introduced 3-D pore structure of various dimensions in a highly reproducible manner. Not only does this make a viable 3-D environment of cells but purely through the control of pore shape, dimension and distribution piezoelectric properties can be induced in the scaffolds, creating a material called a ferroelectret, materials of this variety show exceptionally high piezoelectric coefficients (d_{33}) [1] [2].Furthermore,ceramic-polymer composites were also produced, sodium potassium niobate (KNN) was chosen as it has been proved to be biocompatible and non-cytotoxic while maintaining a high d_{33} of 700 pC/N [3], even when soaked in saline solution its d_{33} is respectable (60-75 pC/N)[4]. Further, like other piezoceramics of its ilk, has shown beneficial effects to cell attachment through polarisation after poling.

RESULTS:The composites were produced through classical regeneration of cellulose using a ceramic-cellulose mixtures of varying compositions (5-80 wt% KNN) and, by the same methodology mentioned above, could introduce similar 3D-pore structure. Regenerated cellulose and its ceramic composites were also tested for cytocompatibility with a neuronal-like cell line (PC-12).

DISCUSSION & CONCLUSIONS:In summary we have successfully produced scaffolds with 3-D internal pore structure of various kinds and proved all our scaffolds to be cytocompatible, overcoming the initial important obstacles in the production of an exciting new active scaffold for tissue engineering applications.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Electroactive materials

Structure and antibacterial activity of zinc and copper-containing hybrid materials

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INTRODUCTION:The use of bioactive glasses for tissue recovery is a very common practice. New formulations are being developed to allow controlled release of clinically beneficial ions. However these materials still exhibit properties very different from those of the tissues where they are implanted. Organic-inorganic hybrids have appeared to overcome this problem. Tetraethyl orthosilicate (TEOS) is usually the inorganic component and polydimethylsiloxane (PDMS) the organic component.
In this work we developed hybrid materials of the PDMS-SiO2-B2O3 system with antibacterial properties due to the addition of zinc and copper.

METHODS:Three compositions were developed, changing zinc or copper amounts and keeping constant the remaining reagents. A different protocol (sol-gel method) was used for each hybrid system.
X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and magnetic nuclear resonance (NMR) were used for structural studies. Microstructure was evaluated by scanning electron microscopy (SEM) and determination of specific surface area (SSA). Degradation tests in water followed the ISO 10993-13 standard (0.5, 1 and 5 hours). Ionic concentrations in the liquid were assessed by inductively coupled plasma optical emission spectrometry (ICP-OES). Antibacterial assays were performed according to the ASTM E2149 method.

RESULTS:Spectroscopic analysis confirmed the presence of hybrid bonds, due to the existence of D-Q bonds between the organic and the inorganic phase, as well as borosiloxane (B-O-Si) bonds. 1H NMR results, together with the XRD analysis, led to the conclusion that zinc-containing samples had shorter PDMS chains, while in copper-containing samples longer PDMS chains were present. 11B NMR analysis showed that in copper-containing samples boron was present in the trigonal form (BO3) while in the zinc-containing samples a change from trigonal (BO3) to tetrahedral (BO4) coordination occurred. Microstructural studies revealed that unlike copper-containing samples, zinc-containing specimens have a porous structure with a much larger SSA. Degradation tests confirmed the ability of the materials for a controlled zinc and copper release. Antibacterial activity for Escherichia coli ATCC 25922 and Staphylococcus aureus NCTC 6871 bacterial strains was observed.

DISCUSSION & CONCLUSIONS:Structure and microstructure of the hybrids was affected by the preparation methodology. All compositions gave rise to stable monolithic samples capable of releasing zinc and copper and exhibiting antibacterial activity against Ecoli and Saureus.

ACKNOWLEDGEMENTS:This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement.

Keywords: Polymers - natural / synthetic / responsive, Other

Combinatorial analysis of tag peptides for enhancement of cell-adhesion short peptides

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INTRODUCTION:For higher bio-compatibility of medical implants, functionalization of their surface to enhance their regenerative performance, balancing the objective reaction enhancement and the unwanted reaction reduction, is an effective approach. Especially with the medical implants for circulatory system, surface modification and its effect have critical life-threatening effect.
Our group have been reporting short functional peptide peptides (3-9) with cell-selective adhesion [1] to support such regenerative balance in the implanted region. Compared to large proteins extracted from animal-derived tissues, short peptides can offer higher purity and productivity by their chemical synthesis. However, one of the disadvantages in short peptides are the difficulty in immobilizing them with their original sequence potency. The length or methods of immobilization can critically decrease their function. Our group had been working on finding good combinational match between the short peptides and its immobilized support, and have found that closely neighboring physicochemical property control is important [2]. We here report our comprehensive combinational effect of short tag peptides on the target short functional peptides, the cell-adhesion peptides, on the peptide array system.

METHODS:For peptide analysis, peptide and tag peptide conjugated library was designed by SPOT peptide array technique on cellulose membrane by conventional Fmoc chemistry. The target peptides' bio-function was assayed directly on the peptide array. We compared several cells on the same peptide-tag combination, and comprehensively compared the effects of short peptide sequences.

RESULTS:From the combination study, it was found that there were critically effective peptide function enhancement effect even by single amino acid, and such combinational importance was confirmed for controlling cell adhesion.

DISCUSSION & CONCLUSIONS:Our result indicated that the immobilization of short functional peptides is critically affected by their neighboring sequences. This result leads to a simple and cost-effective surface peptide functionalization concept for better biocompatible medical implant surfaces.

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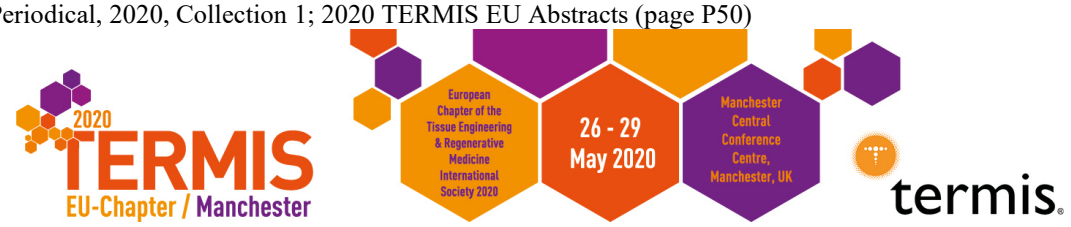
Keywords: Interfaces - biological, Enabling technologies



A comparative chemical, physical and biological evaluation of Spider (Linothele megatheloides) and silkworm (Bombyx mori) silks

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INTRODUCTION:Spider silks have been paid attentions for decades, mainly due to their unique and remarkable mechanical properties[1]. Recently, the study of considering spider silks as possible candidates for biomaterials are becoming popular. In this work, for the first time, the silk from a Colombian spider, Linothele megatheloides (LM)[2], which produces a single type of silk in a relatively large amount, was studied in comparison with silk from Bombyx mori silkworm, before and after degumming, with the evaluation of their chemical, mechanical and biological properties, to explore its use for tailored tissue engineering applications[3].

METHODS:Fibers produced by LM spider and silkworm were evaluated in terms of chemical (amino acid composition, secondary structure analysis) and physical properties (surface adhesion energy, tensile test and morphology observation). Early cell adhesion, metabolic activity and proliferation were investigated by culturing NIH 3T3 cells (normal murine embryo fibroblasts) on the different silk fibers and networks. The results were assessed by imaging (confocal microscopy and FE-SEM) and quantitative analysis (AlamarBlue and PicoGreen assay).

RESULTS:Compared with silkworm silk, LM silk presented higher amount of Serine and lower content of Glycine and more long chain residues in amino acid residues compositions. For protein secondary structure, LM fiber showed the lowest β-sheet and α-helix structures with the highest random coils. The single fiber size of LM was much smaller and the spontaneous assembling of silk filaments was different. The energy at break of LM silk was higher but with broader distributions in Weibull distributions of the strength. LM silk had the highest level of surface adhesion energy among all the others as well as roughness. In vitro test, cells were seen to adhere faster and in a larger amount with a higher specific activity, when in contact with LM fibers. A continuous cell monolayer was observed after two days' culture on LM silk.

DISCUSSION & CONCLUSIONS:For the first time, LM spider silk was selected, charaterized and compared with the well known silkworm silk. The biological behavior of LM silk in vitro test (preliminary) justifies further and deeper investigations to explore its use for tailored biomaterial for tissue engineering scaffolds.

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Keywords: Biomaterials, Biomaterials

Fabrication of a polymeric vascular scaffold that enables to study angiogenesis at cellular and tissue levels

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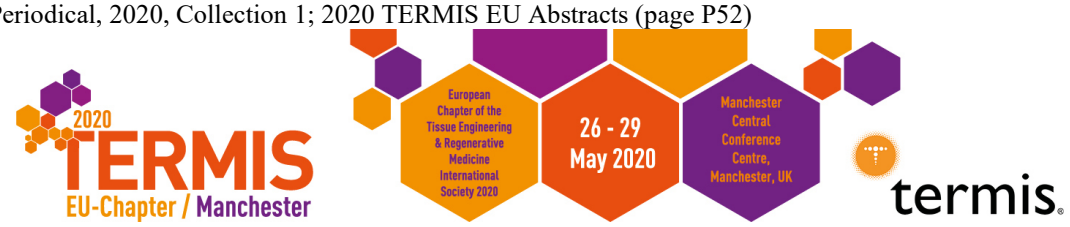
INTRODUCTION:Angiogenesis assays are essential for studying the aspects of angiogenesis and for the discovery of new drugs. To date, there is an extensive collection of in vitro and in vivo angiogenesis assays that are widely used in angiogenesis-related studies. Although in vivo assays are the most representative of the native angiogenesis, they are ethically questionable, require technical skills, and expensive. On the other hand, in vitro assays are inexpensive and relatively easy to perform, but the majority of them are in an only two-dimensional cellular level, which lacks the physiological relevance of three-dimensional structure. Thus, it is crucial to look for alternative platforms to study angiogenesis under more physiologically relevant conditions in vitro.

METHODS:In this study, we used Poly3-hydroxybutyrate-co-3-hydroxyvalerate for the production of synthetic vascular networks combining electrospinning and 3D printing techniques, and revealed the potential of the developed SVN to be used for the study of angiogenesis and for vascularisation of a reconstructed human skin model in vitro.

RESULTS:Our results suggested that the developed platform enabled the study of more than one aspect of angiogenesis, endothelial migration and tube formation, in vitro when combined with Matrigel. We successfully reconstructed a human skin model, as a representative of a physiologically more relevant and complex structure, and assessed the suitability of the developed in vitro platform for studying endothelialisation of the tissue-engineered skin model.

DISCUSSION & CONCLUSIONS:We successfully fabricated vascular scaffolds combining electrospinning and 3D printing. The developed platform enabled the study of angiogenesis at cellular and tissue levels. With further improvements this model could offer a great platform for angiogenesis users to reduce the use of animals in angiogenesis research.

Keywords: Biomaterials, Vascular systems / vascularisation and heart



A frugal innovative approach for abdominal wall repair: characterization of low-cost synthetic meshes

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INTRODUCTION:Abdominal hernia surgery involves the use of synthetic or animal-derived materials to reinforce defects within connective tissue in the abdominal wall. However, these materials are expensive and not affordable in low-income countries so surgeons operating in these areas found nylon mosquito net as a more frugal alternative that has already shown comparable outcomes to commercial meshes [1]. There is great variability and little characterisation of mosquito net mesh so the aim of this study is to characterise the mechanics, ultrastructure and biocompatibility of large (M-LP) and small (M-SP) pore nylon mesh, to assess their suitability for widespread use.

METHODS:Break stress, break strain and Young's Modulus of M-LP and M-SP were obtained with a uniaxial tensile test (BT1-FR5.0TN, Zwick Roell Group, Ulm, Germany). Ultrastructure was evaluated with a Scanning Electron Microscope (FEI UK, UK) operating at 5 kV. Viability of Human Dermal Fibroblasts (HDFs) cultured in DMEM was assessed with LIVE/DEAD® Viability/Cytotoxicity Assay kit (Thermo Fisher Scientific - Life Technologies).

RESULTS:M-SP showed a significantly higher tensile strength (13.77 ± 0.66 N/cm) than M-LP (11.02 ± 0.33 N/cm) ($p < 0.05$) but significantly lower extensibility (110.3 ± 3.4 %) than M-LP (130.5 ± 6.3 %) ($p < 0.0001$). Young's Modulus of M-SP was not significantly different from that of M-LP (17.4 ± 1.2 N/cm and 18.7 ± 2.3 N/cm respectively). Data were analysed with unpaired t-test (N=8). Ultrastructural analysis showed that both meshes are multifilament and M-SP has smaller pores than M-LP. Viability tests, cellular surface area and elongation showed no significant difference between HDFs cultured in DMEM and mesh-conditioned media. HDFs were also cultured on the meshes showing good attachment and proliferation to mesh fibres. DISCUSSION & CONCLUSIONS:Values of break stress, break strain and Young's Modulus of M-LP and M-SP were compared to those of rodent abdominal wall tissue [2]. Low-cost meshes had higher extensibility than native tissue, but similar stiffness and tensile strength demonstrating affordable alternative for hernia repair [2]. Moreover, this is the first study assessing biocompatibility of HDFs in direct contact with low-cost meshes and their attachment.

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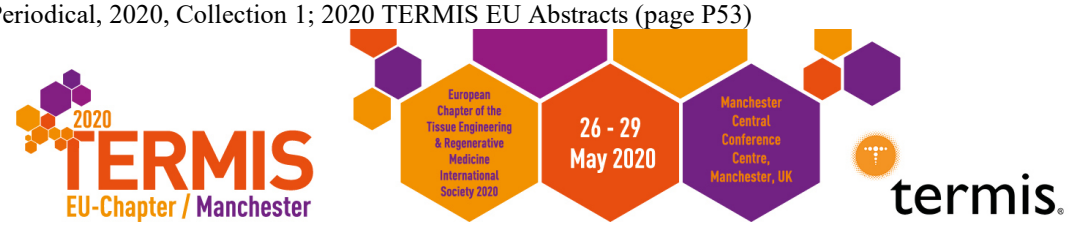
Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc),



Biocompatible tropoelastin-based composites with tunable microstructures and tailored mechanical properties for small-diameter vascular grafts

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INTRODUCTION:Occlusive artery disease is a leading cause of death worldwide. Autologous blood vessels are gold standard replacements but have limited availability. Commercially available synthetic small-diameter vascular grafts (VGs) are susceptible to occlusion due to thrombosis and intimal hyperplasia and fail to promote artery regeneration. To overcome these limitations, biodegradable and biocompatible materials with matched mechanical properties to native arteries are required. Here we describe the development of small-diameter VGs comprising composite constructs utilizing recombinant human tropoelastin and a biodegradable elastomer.

METHODS:The microstructures of the tropoelastin-elastomer composites were assessed using scanning electron and multiphoton microscopy. Mechanical properties were obtained by tensile testing to derive ultimate tensile strength (UTS) and Young's modulus (E). The stability of the composites was tested through long-term mass degradation. Composites were cultured with vascular endothelial cells (ECs) and smooth muscle cells (SMCs) in vitro and proliferation, morphology, and vascular-related gene expression were determined. In vivo biocompatibility was assessed by mouse implantation studies.

RESULTS:A spectrum of composite microstructures was obtained. Incorporating less tropoelastin resulted in fiber-reinforced matrix structures, which with increasing tropoelastin content transitioned into fibrous structures. These composites showed tailored UTS ranging from 0.7-8 MPa and E ranging from 1-26 MPa. The composites retained > 93% mass after 154 days with no evidence of leached tropoelastin. We found that tropoelastin significantly enhances the proliferation of ECs and SMCs in vitro and vascular-related gene expression. Compared to elastomer alone, reduced inflammatory responses in vivo were seen for tropoelastin-containing composites, which were accompanied by decreased inflammatory area thickness and controlled cell numbers surrounding the composites.

DISCUSSION & CONCLUSIONS:Fabricating techniques for tissue-engineered small-diameter VGs have been intensively explored, but most fail to progress towards clinical applications. The unmet need is to develop VGs that have adequate mechanical properties and promote artery regeneration. Tropoelastin, the soluble precursor of elastin, is attractive because it is responsible for the elasticity of the human arteries, and in the fabricated composites. With adjustable degradation processes of the elastomer in vivo, the tropoelastin content is gradually exposed to vascular cells and can promote de novo elastin formation with the potential to reproduce the complex architectural organization of artery elastin. In conclusion, we have combined tropoelastin with a biodegradable elastomer, obtained biomimicking composite materials with an attractively tunable 3D microstructure, matched mechanical properties to the human coronary artery with excellent biocompatibility, and generated promising candidates for off the shelf small-diameter vascular grafts.

Keywords: Cardiovascular, Vascular systems / vascularisation and heart

Innovative platform for the development of α-chitin-based 3D constructs using biocompatible ionic liquids

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INTRODUCTION:Chitin has been widely used for the development of therapeutic strategies, despite its challenging processing. Ionic liquids (ILs), emerged as solvents, able to provide a platform for its effective processing [1]. However, the most common ILs are not as green as thought, and biocompatible ILs (Bio-ILs) arise as bio-friendly alternatives, with enhanced toxicological features [2].

METHODS:Herein, was evaluated the ability of a Bio-IL, Choline Acetate ([Ch]Ace), for the processing of α-chitin into 3D structures. α-chitin was dissolved in [Ch]Ace using ultrasound and stirring cycles at 90ºC. The solution was moulded, kept at -20ºC overnight and immersed in solvents (ethanol, acetone, 2-propanol, and methanol), to modulate the properties of the gel. The samples were frozen at -80ºC and freeze-dried. Their morphology was evaluated by SEM, and the stability by immersion in PBS and H2O. Two formulations were established considering the presence (A) or absence (B) of the Bio-IL into the structure, which was monitored via conductivity measurements. The microstructure of the sponges was determined by µ-CT. The cytotoxicity and biological behavior of the sponges were evaluated using the L929 cell line during 3 days of culture, and human adipose stem cells (hASCS) up to 7 days of culture, respectively, both by direct contact assays.

RESULTS:The obtained optical microscopy images demonstrated that the dissolution of chitin in the Bio-IL was attained after 4 cycles. The SEM analysis revealed that the sponges present an open structure (15-45µm). The stability test showed that the most stable structures were obtained when using methanol, and differences in the conductivity of the solvents confirmed the different Bio-IL content in the formulations (A and B). The µ-CT results revealed porosities of 72.33±1.15% and 85.77±1.93% and pore size values of 173.43±28.54µm and 176.70±44.48µm, for the formulation A and B, respectively. The cytotoxicity test allowed to prove the non-cytotoxic profile of the constructs, as well as the harmless effect of the Bio-IL in the cell metabolic activity. Moreover, hASCS cells were able to adhere, grow and proliferate through the structures, up to 7 days of culture.

DISCUSSION & CONCLUSIONS:The presented approach opens new avenues to develop α-chitin-based sponges using Bio-ILs with potential biomedical applications.

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Keywords: Polymers - natural / synthetic / responsive

Poly(2-hydroxyethyl methacrylate) (PHEMA)-based hydrogels as a synthetic platform to study adipose-derived stem cell (ASC) regenerative potential

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INTRODUCTION:Poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogels are well-studied materials for tissue engineering due to their biocompatibility, controlled synthesis and their ability to tailor their physicochemical properties [1]. Gelatin methacrylate (GelMA) is a semisynthetic material that retains cell-recognition sites, undergoes enzymatic degradation, and has good mechanical properties [2]. Adipose-derived stem cell (ASCs) have shown potential in aiding the regeneration of peripheral nerves as their secretome influence axonal regeneration [3]. This work shows the synthesis and characterization of PHEMA-based hydrogels as platforms to study the influence of ASCs in nerve regeneration.

METHODS:Hydrogels based on HEMA, glycerol monomethacrylate (GMA) and GelMA were synthetized via redox-initiated free-radical polymerization and their mechanical properties were assessed using oscillatory rheology and swelling studies. The ability of ASCs to attach and proliferate was assessed, as well as their overall regenerative performance.

RESULTS:Homogeneous hydrogels with storage moduli in the range 8-12 MPa were prepared using initial water concentrations between 30-40 % w/w, with respect to monomer. Liquid uptakes up to 200 % were observed whilst bulk dimensionalities were maintained. The incorporation of GelMA resulted in stiffer hydrogels, when compared to those containing only HEMA and GMA. GelMA containing gels displayed sustained losses in mechanical properties after incubation in collagenase solution, showing a 5 MPa decrease in storage moduli and increases in their liquid uptake profile after 10 days. LIVE/DEAD and morphological assessments have shown potential for ASC maintenance.

DISCUSSION & CONCLUSIONS:These PHEMA-based hydrogels are ideal platforms for tissue engineering applications due to their ease of preparation and tailorable properties. In this work, hydrogels with mechanical properties in the range of 8-12 MPa have been shown to enhance the neurotrophic potential of ASCs [4]. The introduction of GelMA appears to be beneficial for nerve regeneration, as degradation of hydrogels is key for tissue regeneration applications [5]. The aforementioned hydrogels are envisioned to be used as synthetic platforms to investigate the role of ASCs in peripheral nerve regeneration and their suitability as artificial nerve conduits.

Acknowledgements:Financial support received from EPSRC/MRC Centre For Doctoral Training in Regenerative Medicine (studentship grant number; EP/L014904/1)

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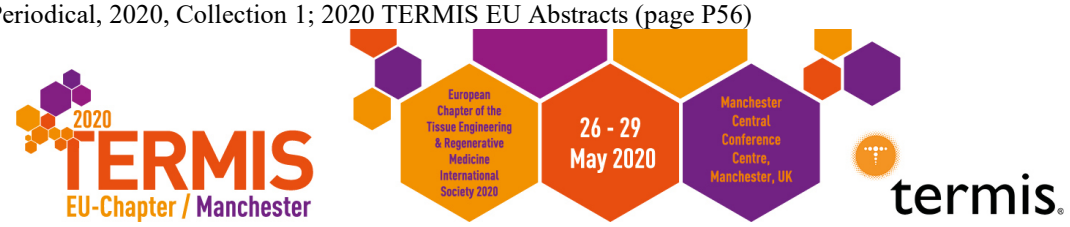
Keywords: Polymers - natural / synthetic / responsive, Hydrogels and injectable systems



Processing SAIB with ionic liquids to produce 3D structures

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INTRODUCTION:Sucrose acetate isobutyrate (SAIB) is a synthetic disaccharide with exceptional rheological properties. It has been lately investigated for drug delivery (WO96399951)1. Nevertheless, the demand for organic solvents to explore the mechanism of solvent diffusion from the depots formation2-4 is a considerable disadvantage. Thus, the use of natural sources together with the sustainable green process is a rational and sustainable approach to produce multifunctional biomedical devices. Herein, it is proposed to explore the potential of SAIB in tissue engineering scaffolding applications, by green processing SAIB with chitin and silk fibroin, using ionic liquids (ILs).

METHODS:SAIB, chitin and silk fibroin were separately dissolved in the IL, and then mixed by adding the SAIB/IL solution to the chitin/IL or SF/IL solution. The mixture was then transferred to moulds (d=8 mm, h=2 mm) and then the produced gels were washed with defined solvents - water (A) or water: isopropanol (1:1, B). These gels were placed in caped flasks at 150 rpm with daily solvent change, and aliquots were collected to measure the conductivity and follow the removal of the ILs. When the IL was totally removed the gels were freeze-dried. The physicochemical characterization performed comprised rheology, FTIR, XRD, SEM, microCT and swelling. The in vitro studies performed covered cytotoxicity, cell damage (ISO10993) and cell morphology.

RESULTS:The presence of the molecules (SAIB, chitin and SF) and the absence of ILs, was confirmed by FTIR and XRD techniques. Using SEM and microCT methods it was possible to observe the morphological characteristics of the produced scaffolds, that resembles different morphologies, from aerogels to compact structures. The presence of SAIB increased about 4 times the adhesivity (N.s) of the scaffolds and, at the same time, has halved the strength of the structure (G'/Pa).

DISCUSSION & CONCLUSIONS:It is shown the potential of the used approach, to apply these scaffolds in tissue engineering.

Acknowledgements:Financial support was received from FCT (SFRH/BPD/94277/2013, SFRH/BPD/100957/2014 and IF/01285/2015) and NORTE-08-5369-FSE-000044.

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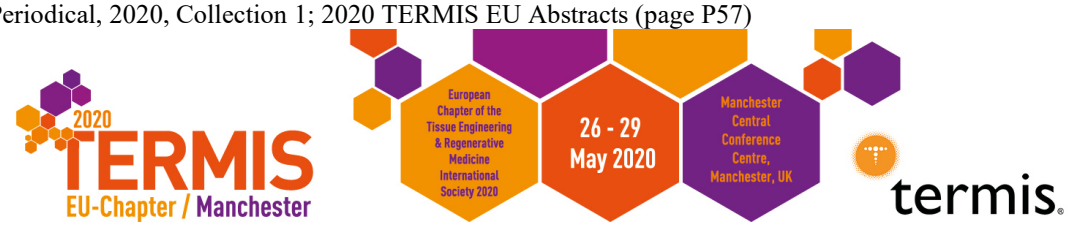
Keywords: Biomaterials, Bioadhesives



A low-temperature, high-pressure sintering procedure for the rapid fabrication of biosubstrates starting from dry silk fibroin

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INTRODUCTION:Due to the unique combination of properties such as mechanical strength and toughness, biocompatibility, biodegradability, thermal stability, and easy processability [1,2], regenerated silk fibroin has been used as a functional biomaterial, adopted when a positive interaction with living tissue is required. Solid-fibroin is a bulk non-porous material that is usually prepared starting from a fibroin solution through a liquid-gel-solid transition. In this work we demonstrate the posibility to produce, in fast fashion, monoliths of solid-fibroin starting from a dry fibroin powder, in presence of water and under fast compression.

METHODS:A full factorial design of experiment was used to understand the sinterign rpocess. We studied the material in the crucial phases of the procedure by DSC, FTIR and SEM, proving that the thermal-reflow can occur at low temperature if driven by a high-pressure process and in presence of water on a low crystallinity dry fibroin. Mechanical characterization and preliminary in vitro tests were conducted: human adipose-derived mesenchymal stem cells were cultured on both LTS fibroin samples and PCL samples (control), to evaluate cell response. After each time point, cell adhesion, morphology and distribution were analyzed by confocal microscopy.

RESULTS:The analysis conducted with FTIR on the main stages of the process revealed that an excessive transition to β–parallel structure, due to a prolongated water treatment, doesn't allow the transition to the solid state; the transition occurs if the secondary structures are not enough stable to be able to re-organize themselves in more stable phases during the compression phase. The glass transition temperature was detected by DSC. Interestingly, the rapid addition of water in the material through moisture absorption didn't change the Tg respect the lyophilized silk fibroin. The SEM analysis during different time points in the compression proved the presence of a viscous flow, so we could deduce a decrease in the Tg under 40 °C when moisturized lyophilized fibroin undergoes to compression. Preliminary biological results indicate a promising response of LTS fibroin samples in promoting cell adhesion and proliferation.

DISCUSSION & CONCLUSIONS:In this work we reported a method to obtain a compact material form fibroin powder in a single compression mold step at low temperature. Large objects can be produced in few minutes with a high reproducibility. The mild forming conditions allow the possibility to incorporate temperature degradable bioactive additives.

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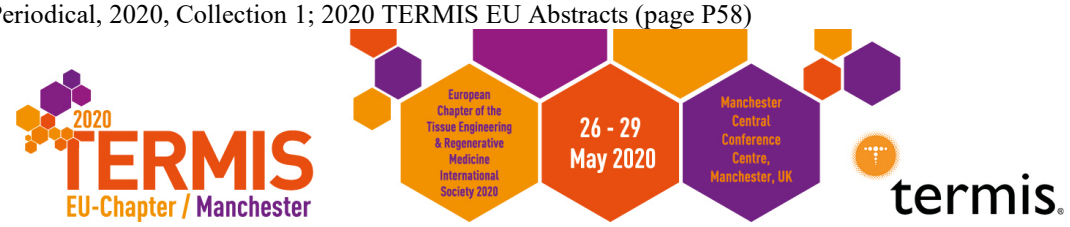
Keywords: Biomaterials, Polymers - natural / synthetic / responsive



In-vitro degradation study of hybrid polyester-based scaffolds in different culture chambers

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INTRODUCTION:Biomaterials play a critical role in the success of a Tissue-Engineering (TE) strategy [1], as well as scaffolds' degradation. Testing this degradation in different culture chambers can lead to different results [2]. This work aims to compare the degradation of 3D-printed composites based on poly(ε-caprolactone) (PCL) and poly(D,L-lactic-co-glycolic) acid (PLGA) in simulated physiological conditions by using standard multi-wells or bioreactors to evaluate if the different culture chamber used affects the degradation's kinetics.

METHODS:PCL-PLGA blend (50:50 wt.) was prepared by dissolving PCL and PLGA in dichloromethane, casting and evaporating the solvent. We extruded filaments with an in-house mechanical extruder, and 3D-printed scaffolds (diameter 6mm, height 2mm, porous 400µm) by plotting fibres (diameter 400µm) with 45° angle-steps between two successive layers. Scaffolds were sterilised by UV irradiation, soaked in Phosphate-Buffered Saline (PBS), and incubated at 37°C for 14days. Polymers degradation was monitored by measuring the weight-average molecular weight (Mw), average molecular number (Mn), polydispersity index (PI), weight loss (WL) and indirectly by the changes in PBS pH. We compared the degradation in standard multi-wells with the ones occurring in a customised bioreactor (CANDY) and the MechanoCultureTR (MCTR) (CellScale, Canada). We used incubated dry scaffolds as control.

RESULTS:From the initial value (7.25), the pH decreased substantially at the early stage (day 2) and stabilised around 6.85. Scaffolds in PBS showed significantly higher WL than the controls. We observed a statistical difference (Welch's t-test) between the WL occurring in CANDY (lower) and the ones (higher) in MCTR and standard multi-wells. Even though Mw, Mn, and PI showed no statistical difference between the different chambers, the results in the MCTR showed a higher Mw/Mn decrease and a PI increase.

DISCUSSION & CONCLUSIONS:The initial pH reduction observed can be due to the degradation by hydrolysis of the ester linkages in PCL and PLGA under the simulated physiological conditions. The pH is acidified but still suitable for biological applications [3]. These results show that the surface of the incubation buffer (multi-wells and partially MCTR) exposed to the air can affect the degradation process. Future studies should consider this effect when comparing degradation results from different culture chambers.

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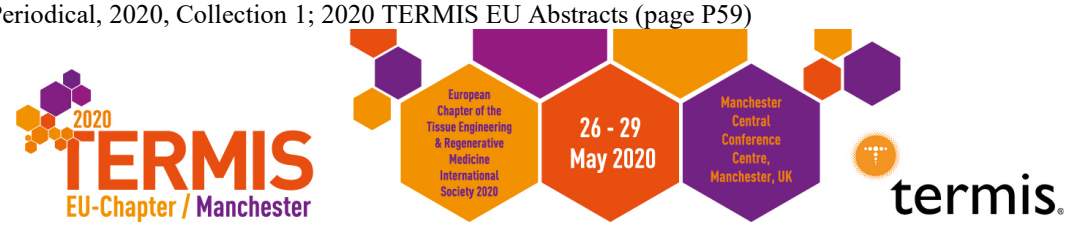
Keywords: Bioreactors, 3D printing and bioprinting



Mechano-Hybrid-Scaffolds for endochondral healing of bone defects

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INTRODUCTION:During the last years, various strategies have been developed to promote bone defect regeneration utilizing implantable materials. Collagen scaffolds are well known for their regenerative potential and are bio-mimicry of early callus formation. However, due to their relatively poor mechanical properties, their applications in the field of bone regeneration are limited. In this study, we present a novel approach: A Mechano-Hybrid-Scaffold (MHS) that combines a collagen-based biomaterial with highly aligned channel-like pores with a 3D printed poly(ε-caprolactone)(PCL) support structure [1], overcoming contradictory requirements for mechanical stiffness and scaffold architecture on the cell vs. the tissue length-scale that are defined by the particular clinical application.

METHODS:Using a standardized manufacturing process including directional freezing the existing orientated collagen scaffold [2] (stiffness E= 0.5kPa) was combined with a 3D printed PCL macro-porous support structure designed to reinforce the scaffolds mechanical stability. In brief, MHS were produced by: 1. Computer-aided design of the support structure (2 designs for high stiffness “stiff” and low stiffness “soft”); 2. Selective laser sintering from PCL; 3. Immersion of the support structure into the collagen dispersion, unidirectional freezing and freeze-drying; 4. Chemical crosslinking and sterilization of the MHS. Structural characterization (SEM) and mechanical characterization (BOSE-Electroforce-test -ench) were performed. Degradability of the MHS was assessed by determination of the denaturation temperature of the collagen (Td).

RESULTS:Stiff and soft MHS were successfully produced with stiffness of 41.2 ± 1.4 N/mm and 0.1 ± 0.02 N/mm for the supporting structure respectively. Except for the mechanical properties of the MHS, all other scaffold characteristics, e.g. Td (79.8°C ±0.1) and pore size (78.1 µm ± 18.1), remain the same as the ones of the collagen scaffold. Scanning electron microscopy (SEM) images show full integration of the support structure inside the collagen structure. With this approach, mechanical characteristics can be tuned independently at the micro-scale (cell-level) and the macro-scale (tissue-level).

DISCUSSION & CONCLUSIONS:The here-introduced concept takes full advantage of the regeneration potential of collagen scaffolds by implementing a second macro-porous structure that provides mechanical support against cell and tissue forces in combination with clinically applied bone fixation systems. The MHS opens the door for new applications of collagen scaffolds in rigid tissue regeneration by solving the paradox of providing soft cell environment and high structural stability in implantable materials.

ACKNOWLEDGEMENTS:The authors acknowledge financial support by the German Federal Ministry of Education and Research (grants no. 13XP5048A-D).

REFERENCES:[1]Patent pending: DE102016007931.2; PCT/DE2017/000183
[2]Petersen et al. (2018), Nat. Commun. 9:4430.

Keywords: Bone and bone disorders (osteoporosis etc), Composite materials

Enhancing the osteogenic and angiogenic activity of the multiscale porous, synthetic scaffolds by in vitro generated extracellular matrix decoration

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INTRODUCTION:Synthetic polymers are widely used biomaterials for tissue engineering applications due to having tailorable properties. However, they need modifications to improve their limited interaction with biological tissues.
In this study, we aimed to manufacture multiscale porous biohybrid scaffolds to be used as bone tissue engineering scaffolds. We used two-step fabrication route; (i) manufacturing of biodegradable, synthetic polymer-based multiscale porous scaffolds by combining techniques of emulsion templating and 3D printing techniques, and (ii) decorating these scaffolds with in vitro generated extracellular matrix to increase their biological performances. The biohybrid scaffolds were evaluated for their ability to support cell attachment, metabolic activity, and osteogenic differentiation by seeding of human embryonic stem cell-derived mesenchymal progenitor cells (hES-MPs). The angiogenic potential of the multiscale porous scaffolds was assessed in vivo.

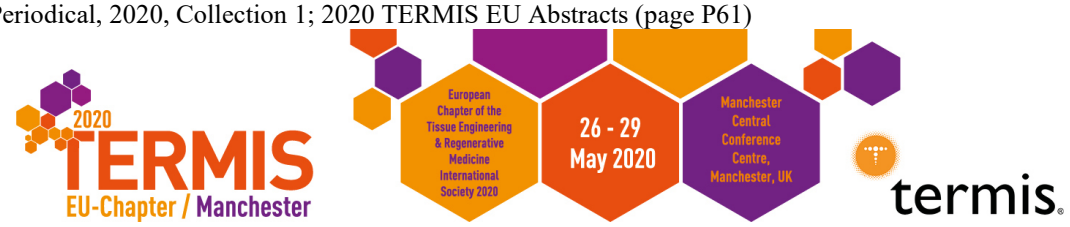
METHODS:Scaffolds, made of high internal phase emulsion (HIPE) of methacrylated PCL (photocurable), were created via additive manufacturing. Murine Osteocyte-like Cells (MLO-A5s) were cultured on scaffolds for 4-weeks. Cellular activity, mineral and collagen deposition assays were performed. Cell penetration depth was assessed by histology images. Microarchitecture of scaffolds and cell attachment were observed by scanning electron microscopy. After decellularisation, to confirm the efficiency of the process, DNA content was measured. Both plain and hybrid scaffolds were recellularisated by using hES-MPs and compared. Finally, the chick allantoic membrane (CAM) assay was used to explore the angiogenic potential of the scaffolds.

RESULTS:Multiscale porous scaffolds were obtained successfully. Cell-derived ECM matrix composed of mineral and collagen was deposited on scaffolds, and then 95% of DNA was removed (decellularisation). In recellularisation process, mesenchymal progenitor cells not only showed higher attachment on ECM decorated scaffolds but also exhibited a significantly higher increase in cellular activity and cell infiltration. Additionally, CAM assay showed that the presence of ECM on scaffolds resulted in higher angiogenic potential in comparison with the plain scaffolds.

DISCUSSION & CONCLUSIONS:3D printed, PCL-based polymerized HIPE scaffolds supported cell adhesion, proliferation and migration, and it is a promising candidate to fulfil the requirements of defect-matching bone grafts. Additionally, our in-vitro generated ECM decorated polymer system proposed an applicable approach to improving bioactivity and angiogenesis of polymer scaffolds to encourage precursors to differentiate mature bone.

Acknowledgements:Authors gratefully acknowledge the Republic of Turkey, The Ministry of National Education for funding Betül Aldemir Dikici with a PhD studentship grant.

Keywords: Additive manufacturing, Decellularised matrices



Bifunctional membrane for guided bone regeneration: combining electrospinning and emulsion templating

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INTRODUCTION:Guided bone regeneration is a dental implant treatment where a barrier membrane (BM) is used between epithelial tissue and bone graft to prevent the invasion of the fast-proliferating epithelial cells into the defect site to be able to preserve a space for infiltration of slower-growing bone cells. Our aim was to develop a bilayer biodegradable BM that would act as a physical barrier for preventing epithelial invasion limiting the diffusion of waste and nutrients, and which would guide bone formation by ingrowth into a highly porous and interconnected bone-like structure.

METHODS:In this study, a bilayer BM was developed by combining electrospinning and emulsion templating. For the first layer, 200 µm polymerised high internal phase emulsion (PolyHIPE) scaffold made of photocurable polycaprolactone (PCL) was manufactured and treated with air plasma to enhance the cellular infiltration. For the second layer, four solvent compositions were investigated to find the best composition for electrospinning a nanofibrous PCL barrier layer on PCL PolyHIPE. The biocompatibility and the barrier properties of the electrospun layer were demonstrated using human dermal fibroblasts (HDFs) over four weeks in vitro by histological staining. Following in vitro assessment of cell viability and cell migration of Murine Osteocyte-like Cells (MLO-A5s) on layer one, the potential of PCL PolyHIPE for supporting blood vessel ingrowth was investigated using an ex-ovo chick chorioallantoic membrane assay (CAM).

RESULTS:The bifunctional BM was successfully produced via emulsion templating and electrospinning. Our results demonstrated that the nanofibrous PCL electrospun layer was capable of limiting cell infiltration for at least four weeks, while PCL polyHIPE supported cell infiltration, calcium and mineral deposition of bone cells, and blood vessel ingrowth through pores.

DISCUSSION & CONCLUSIONS:This novel bioresorbable membrane has the potential to be used in GTR applications by acting as a barrier for epithelial cells at least for up to 4 weeks while allowing bone cells to grow and blood vessels to infiltrate in the implant zone.

Acknowledgements:Authors gratefully acknowledge the Republic of Turkey, The Ministry of National Education for funding Betül Aldemir with a PhD studentship grant.

Keywords: Biomaterials, Bioreactors

Investigation of Endothelial Cell Viability and Growth on 3D Printed GelMa Vascular Networks

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INTRODUCTION:A limitation for the development of 3D engineered tissues is the absence of viable and perfusable vasculature [1-3]. As a precursor to vascularized adipose tissue, cylindrical channels were formed in a cast photocrosslinked gelatin methacrylate (GelMA) construct by printing sacrificial networks of Pluronic F127. Human umbilical vein endothelial cells (HUVECs) were seeded and cultured within the 3D printed channels, while Adipose derived stem cells (ADSCs) were cultured in the GelMA prior to casting the 3D printed channels.

METHODS:GelMA was synthesized using the one pot synthesis method [2]. The hydrogel was characterized by NMR, surface tension, contact angle, DMA and rheology. Pluronic filaments were printed onto glass slides using a robotic printer I&J 7300-LF (Fishnar, UK). HUVECs (PromoCell, UK) were cultured on GelMa substrate, whilst ADSCs (ThermoFisher) were embedded within the GelMa. Live/Dead and Alamar Blue assays were used to assess the cells' viability and proliferation respectively. Phalloidin staining was used to assess actin cytoskeleton organization. Further experimentation included the differentiation of the ADSCs into adipocytes onto GelMa.

RESULTS:Once methacrylation has occurred, NMR peaks are seen at 6ppm and 2ppm corresponding to lysine and methacrylated grafts of hydroxyl groups. Viability assays confirmed that HUVECs and ADSCs were viable after 7 days. Phalloidin staining demonstrated good organization of the actin cytoskeleton of HUVECs on GelMa. Data on HUVECs injected within the printed 2D networks and 3D culture of ADSCs within the GelMa matrix will also be presented.

DISCUSSION & CONCLUSIONS:Collectively, our data illustrate that HUVECs grow and fully line the printed networks, thus confirming the formation of a vascularized model.

ACKNOWLEDGEMENTS:CC and FZ were supported by studentships from EPSRC and CSC.

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[2] Shirahama H et al. 2016; 6:31036
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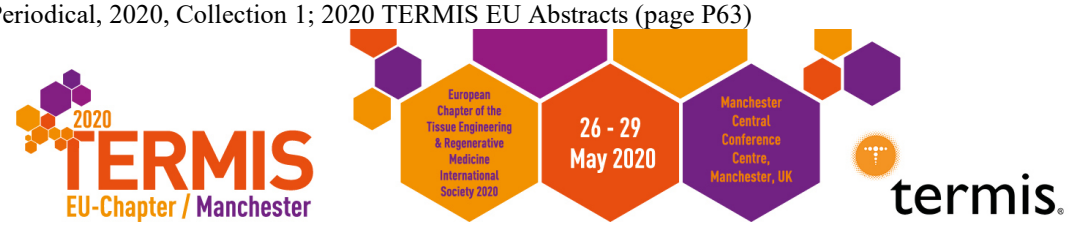
Keywords: 3D printing and bioprinting, Biomaterials



Mechano-electrically active biomaterials for bone tissue engineering

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INTRODUCTION:Active biomaterials, in particular electroactive polymers, have shown increasingly potential to be used in tissue engineering(TE), once they can deliver an electrical signal to the cells upon mechanical solicitation, opening new scientific and technological opportunities, as they in fact mimic signals and effects present in living tissues, allowing the development of suitable microenvironments for tissue regeneration.Piezoelectric polymers have already shown strong potential for novel TE strategies,once they can account for the piezoelectricity existence within some specific tissues.In the case of the patient is immobilized, where the natural mechanical stimulus does not occur, magnetoelectric biomaterials can be used, providing the mechanical and/or electrically stimulation remotely from outside the human body and/or during in-vitro cell culture.
Thus, a novel overall strategy for bone TE was developed based on the fact that bone cells are subjected to mechano-electrical stimuli in their in-vivo microenvironment and that piezo- and magnetoelectric polymers are suitable for delivering those cues.

METHODS:Piezoelectric poly(vinylindene fluoride)(PVDF) films were produced by solvent casting and films with different surface charge are obtained(non-poled and negative).Magnetoelectric films and 3D scaffolds were also produced:magnetoelectric Terfenol-D/poly(vinylidene fluoride-co-trifluoroethylene)(P(VDF-TrFE)) films and CoFe2O4/PVDF 3D scaffolds.
Piezoelectric and magnetoelectric polymers were produced and characterized in order to investigate the influence of the mechano-electrical stimuli and consequently their performance in the novel bone TE development.

RESULTS:hASCs were cultured on β-PVDF non-poled and negative surfaces and cell differentiation was determined using a qALP assay. The results demonstrated that the negative charge promote higher osteogenic differentiation, which is even higher under dynamic conditions(by the use of mechanical bioreactor).´ Terfenol-D/P(VDF-TrFE) films enhanced up to≈25% when cells are cultured under mechanical and electrical stimulation.Through the use of CoFe2O4/PVDF 3D scaffolds, an unequivocal increase of the proliferation rate is observed by the stimuli application,indicating that the scaffolds indeed respond to the magnetic stimuli and provide a proper microenvironment to the cells.

DISCUSSION & CONCLUSIONS:It is thus demonstrated that electroactive biomaterials can provide the necessary electromechanical stimuli for the proliferation and differentiation of bone cells.In this way, this approach support the design of suitable bone TE strategies, showing that piezoelectric and magnetoelectric cell stimulation is a novel and suitable approach that allow the application of magnetic,mechanical and electrical stimuli.

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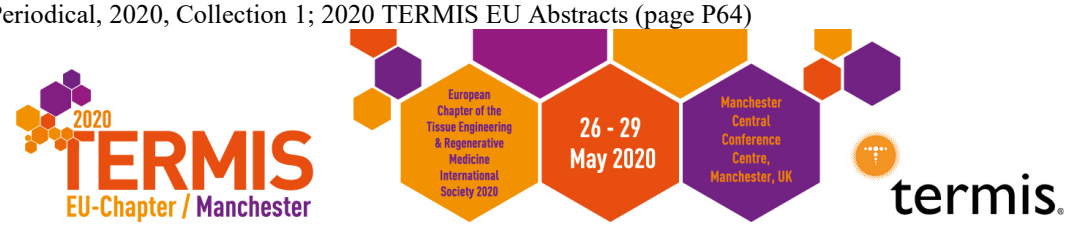
Keywords: Electroactive materials, Bioreactors



Freeze-cast silk fibroin scaffolds for cardiac patch development

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INTRODUCTION:Cardiac patches consist of 3D scaffolds seeded with appropriate cell types, and are a promising avenue towards developing effective treatments for scarred heart muscle. However, the impact of these scaffolds has been limited due to poor mass transfer caused by inadequate scaffold microarchitecture. Freeze-casting is a cryostructuring process that yields scaffolds of uniformly aligned pores. Setups that use volatile heat sinks (e.g. liquid nitrogen) have garnered attention due to their simplicity, but little has been done to characterise the effect of system thermodynamics and polymer concentration on pore morphology. By using silk fibroin as a representative polymer, the goal of this study was to quantify these correlations and design novel methodologies that address the limitations of conventional freeze-casting systems.

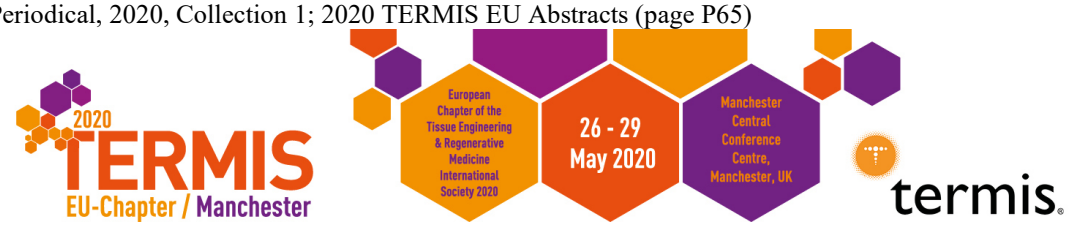
METHODS:Silk-fibroin was freeze-cast using two separate setups. The first (a conventional single unit setup) exposed one side of insulated silk solution material to a metal plate in contact with a dry ice/ethanol slurry (T≅ -60 to-75 °C). The second consisted of a modular design, whereby an insulated silk chamber with a copper base was placed on top of the copper plate of a separate heat sink chamber containing the slurry. The thermal profile was documented along the silk chamber and subsequently correlated to pore morphology.

RESULTS:Every condition yielded scaffolds with uniformly aligned pores that sustained the adhesion, proliferation and alignment of C2C12 cells. The resultant pore morphology depended on both the cooling rate and silk concentration. A decrease in cooling rate resulted in larger pore inlets. Furthermore, an increase in silk concentration resulted in an increase in lamellae thickness and overall pore alignment. While using the single unit setup the mould warped, causing damage and resulting in leaching of the heat sink into the silk chamber; a common problem with similar setups. Whereas the novel modular setup did not display the warping seen in the original. The separation of silk and heat sink chambers also allowed for easy leak monitoring. Additionally, the modular setup provided more controllable cooling profiles.

DISCUSSION & CONCLUSIONS:Characterising the correlation between pore morphology and freezing variables provides the insight required for finer control over pore morphology of freeze-cast biomaterials. The proposed modular design allowed for simpler monitoring, while providing more control over cooling rate. Future studies will utilise heat sinks of different temperatures to quantify the mass transfer potential and test the limits of this design.

ACKNOWLEDGEMENTS:Australian Government Research Training Program Scholarship

Keywords: Cardiovascular,



3D in vitro intestinal mucus models for dynamic culture of bacteria

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INTRODUCTION:Although the intestinal mucus is the main intermediator between microbiota and gut, it is rarely considered as a dynamic component in current in vitro models of the microbiota-gut communication [1]. Recently, the MINERVA project was funded by the European Research Council (ERC, ID 724734) with the aim of developing the first microbiota-gut-brain organ-on-a-chip engineered platform to evaluate intestinal microflora impact on brain functionality [2]. Under the scope of MINERVA, an optimized in vitro mucus model suitable for microbiota dynamic culture was engineered, which combines a 3D structure and proper viscoelastic properties, to be applied in the dynamic organ-on-chip devices developed for MINERVA purposes.

METHODS:Different mucus models were obtained by homogeneous crosslinking of alginate solutions, at different concentrations, with CaCO3 (Sigma-Aldrich, Lot MKBZ9710V) and an acidifying agent (D-(+)-gluconic acid δ-lactone, GDL, Sigma-Aldrich G4760 Lot SLBM7762V). Rheology (modular rheometer, Anton Paar) was used as method to investigate the viscoelastic properties of the models after extrusion at different flow rates (0.5, 1.0 and 5.0 µl/min). From the rheology, the models microstructure was further estimated by using the Generalized Maxwell Model. Biological validation of the models was performed by counting of colony forming units after the extrusion of models infected by E. coli.

RESULTS:Models with 1 % (w/v) alginate showed viscoelastic properties compatible to the physiology post extrusion at 0.5 and 1.0 µl/min. The Generalized Maxwell model allowed estimating from the rheology of the model mesh size, which is the distance between the polymeric chains forming the network (20-40 nm). This structural parameter resulted similar to the in vivo situation (30-100 nm). The models were also able to sustain the E. coli growth similarly to standard method of dynamic bacterial culture (~ 〖10〗^7 bacteria/ml), i.e. planktonic suspension.

DISCUSSION & CONCLUSIONS:Homogeneous alginate hydrogels represented the first in vitro approach combining 3D structure and bioinspired viscoelastic properties. The models were able to sustain the E. coli growth even under dynamic condition within a 3D medium-free environment. For these features, the described models can be exploited in the advanced organ-on-a-chip devices developed for the MINERVA platform [2]

ACKNOWLEDGEMENTS:MINERVA project received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement no.724734).

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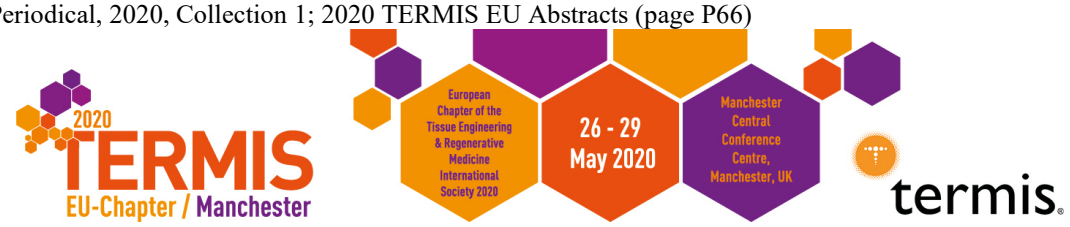
Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, In vitro microenvironments



Preparation of biocompatible oligomers

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INTRODUCTION:Oligomers have potential application for printing and as adjuvants for vaccines. Preparing oligomers is especially worthwhile for stereolithography. So one gets materials in the needed viscosity range. Oligomers are favourable as adjuvants for vaccines, as they facilitate renal clearance. Methacrylic polymers known to be widely tolerated in vivo, so they are widely used in biology and medicine. So, to prepare the oligomers methacrylic monomers were used.

METHODS:The oligomers were prepared by catalytic chain transfer polymerisation (CCTP). Cobalt macrocycles are effective and mostly used catalysts for the CCTP. This is disadvantageous for medical applications, as cobalt is toxic. Iron has higher biocompatibility and is also abundant in nature, so it is additionally more sustainable. The catalysts were prepared in situ. For that iron bromide and dimethyl glyoxime or diphenyl glyoxime were added as ligands. Different catalyst concentrations were used. The reactions were made in bulk and with toluene as solvent

RESULTS:The reaction conditions influenced the level of control. For reactions using diphenyl glyoxime as ligand it was preferable to prepare the oligomers in bulk. In contrast to that dimethyl glyoxime as ligand could control the reactions better in solvent. To control the reactions higher concentration of iron catalysts were needed than in case of cobalt catalysts.

DISCUSSION & CONCLUSIONS:Iron catalysts are a possible replacement for cobalt catalysts for the CCTP. This allows to prepare reproducible, biocompatible oligomers. Further work will be done to optimise the conditions.

Keywords: Polymers - natural / synthetic / responsive, Immunity / immunomodulation / macrophage

Proteomic characterization of calcium-doped sol-gel coatings

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INTRODUCTION:Researchers are carrying out huge efforts to develop the optimal biomaterial that fulfils a particular requirement. Additionally, the poor correlation found between in vitro and in vivo, especially in bone tissue, highlights the need to new characterization methodologies. Bone tissue regeneration around an implant depend on processes such osteogenesis, coagulation, angiogenesis and inflammation. Protein adsorption onto the material surface is the first event-taking place after implantation. These proteins can modulate biological processes are carried out afterwards and condition the implant outcome. The role of calcium on bone metabolism and blood coagulation processes justifies its use in the development of bone tissue engineering biomaterials. Ca can condition the material-tissue interface, affecting protein deposition and consequent cellular responses. This work aims to study, through proteomics, the adsorbed protein patterns onto Ca-doped biomaterials.

METHODS:A previously sol-gel material [1] was employed as release vehicle to incorporate Ca. This matrix was supplemented with 0.25, 0.5, 1 and %wt CaCl2. Ti discs were coated with prepared sol-gels. Physicochemical parameter as topography, roughness, wettability and the Ca2+ kinetic release were determined. In vitro was assessed with MC3T3-E1 osteoblast and RAW 264.7 macrophage cells. Proteomic assay was conducted by incubating the samples with human serum for 3 h. Attached proteins were eluted with SDS-DTT solution. Finally, elutions were analyzed using LC-MS/MS.

RESULTS:The calcium incorporation into the sol-gel increased the coating roughness and decreased their hydrophilicity. In vitro using MC3T3-E1 revealed an overexpression of both osteogenic markers ALP and OPN on Ca-enriched coatings. In parallel, the Ca incorporation supposed an increased expression of anti-inflammatory marker IL10 and a reduction in the release of TNF-α in a dose-dependent manner respect the base material on RAW264.7. Ca-doped materials displayed a remarkably increase on the attachment of coagulation-related proteins (THRB, ANT3, PROC) and a higher affinity to VTNC and APOE, proteins involved on osteogenic and anti-inflammatory functions.

DISCUSSION & CONCLUSIONS:The adsorbed protein pattern movements onto biomaterial surfaces with different calcium amounts were identified. A cluster of proteins related to coagulation and osteogenic functions was identified to improve their affinity to Ca-coatings. Additionally, Ca dose-dependent effects on both osteogenic and inflammatory processes were observed in vitro and confirmed by proteomics.

Acknowledgements:This work was supported by MINECO [MAT2017-86043-R, RTC-2017-6147]; Universitat Jaume I [UJI-B2017-37, POSDOC/2019/28]; Generalitat Valencia [GRISOLIAP/2018/091]; Basque Government [IT611-13, Predoc/2016/10141]; University of the Basque Country [UFI11/56].

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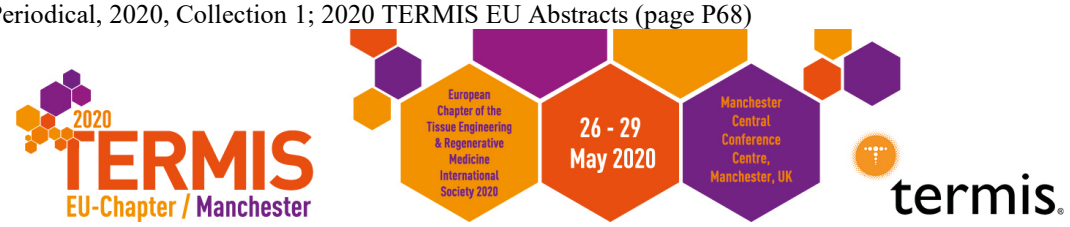
Keywords: Interfaces - biological, Omics / bioinformatics and systems biology



Cytotoxicity of drugs used for covering of vascular stents and drug enriched 3D matrices produced by electrospinning against artery wall cells

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INTRODUCTION:Neointima growth after angioplasty with or without stenting is the main source of restenosis. Adequate inhibition of neointima growth induced by tissue damage during angioplasty requires delicate management of cell proliferation assuming preventing of excessive fibroblasts and myocytes growth while supporting healing and endothelization. To satisfy these demands and provide accurate drugs dosage the precision data on their cytotoxicity is are demanded.

METHODS:Human umbilical vein endothelial cells (HUVEC), human fibroblasts (HF), vascular smooth muscle cells (VSMC), HeLa and HepG2 cells were used to study cytotoxicity of sirolimus (Srl) and paclitaxel (PTX). Cells were cultivated with toxicant for different time intervals and cell viability was studied using Alamar Blue® reagent. Drug enriched matrices were produced by electrospinning (ES) from PCL solutions in HFIP combined with human serum albumin, DMSO and drugs. Matrices (100–130 μm) were characterized by SEM, tensile strength and contact angle. Cytotoxicity of matrices was studied as described in ISO 10993-5-2011 and in a contact test after seeding of the cells onto matrices.

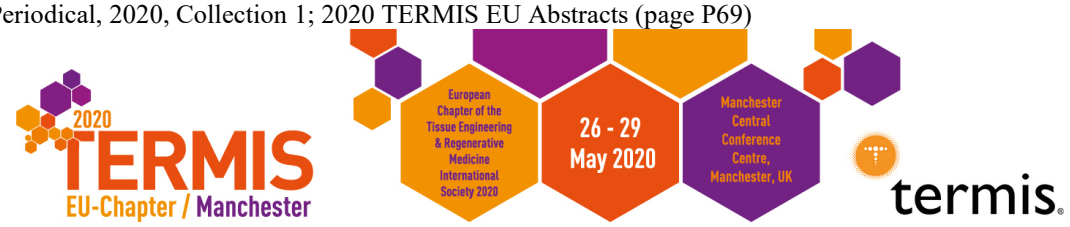
RESULTS:PTX inhibits HUVEC proliferation starting from 0.5 ng/ml, reaching 50% at 5 ng/ml. HeLa and HF cells, 10 times higher concentrations of PTX is required for 50% cell growth inhibition. HepG2 and VSMC slowly grow at 5 ng/ml, however, 50% cell death was observed at a concentration only 500 ng/ml. The cytotoxic effect of Srl is observed at a concentration of 1 ng/ml, however, increasing the concentration to 2 μg/ml does not lead to a significant decrease in viability, which remains at the level of 60-70% for HUVEC and HF, and at the level of 80-90% for HeLa, HepG2 and VSMC.
Based on these data, matrices containing 0.46 μg/cm2 of PTX and 0.92 μg/cm2 of Srl were made. All matrices consisted of fibers varying in diameter from 0.1 to 0.6 μm with a tensile strength similar to previously published data [1]. PTX-eluting matrices reduce proliferation of HF up to 40%, HeLa up to 30% and HepG2 up to 60%. Srl-eluting matrices do not interfere with HeLa and HepG2 proliferation, but reduced proliferation of HF to 60%.

DISCUSSION & CONCLUSIONS:Cytotoxic and suboptimal concentrations of PTX/Srl were specified for drug-eluted ES-produced materials. Drug-eluted matrices demonstrate moderate toxicity and are suitable for production of ES covered vascular stents for in vivo study.

Acknowledgements:The study was supported by RSF grant 18-15-00080.

References:[1] Chernonosova V., et al., Pol. Adv. Tech. 2017; 28:819–27.

Keywords: Vascular systems / vascularisation and heart, Polymers - natural / synthetic / responsive



Development of Anisotropic Gradient Mineralised Hydrogels for Osteochondral Tissue Regeneration

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INTRODUCTION:Osteochondral (OC) tissue interfaces within the knee joint biomechanically integrate cartilage to underlying bone via structural and cellular gradients. Due to the limited regenerative capacity of articular cartilage, OC defects require surgical intervention to restore native joint biomechanics. However, current treatment methods demonstrate limitations. For example, allograft and autograft implantations are limited by donor tissue availability, whilst the regenerative technique of matrix-assisted autologous chondrocyte implantation (MACI) requires a costly cell expansion In-vitro. As a potential alternative to current treatment methods, a recently developed electrophoretic platform has facilitated the production of anisotropic gradient mineralised hydrogels as gene-activated matrices (GAMs) as an in-situ approach to OC tissue regeneration. This novel approach enables the controlled coprecipitation of plasmid-DNA-loaded calcium-phosphate nanoparticles (p-Cap-NP) and plasmid DNA-loaded magnesium phosphate nanoparticles (p-MgP-NP) within an agarose matrix to spatially control nucleic acid drug delivery, biomineralisation and therefore transfection of invading progenitor cells. Monophasic p-CaP-NP matrices, p-MgP-NP matrices, and biphasic p-CaP-NP/p-MgP-NP matrices were prepared using this electrophoretic platform, and their bioactivity and pDNA release in simulated body fluid (SBF) was characterised.

METHODS:Agarose gels were prepared in a buffer of HEPES and Na_2HPO_4, then cast in a horizontal electrophoresis tank for monophasic matrices or in a novel in-house made vertical electrophoresis tank for biphasic matrices. Monophasic matrices were loaded with a single reporter-pDNA and either $CaCl_2$ or $MgCl_2$ solution, whilst biphasic matrices were sequentially loaded with two reporter-pDNAs and both $CaCl_2$ and $MgCl_2$. Matrices were then lyophilised, incubated in SBF and the change in supernatant Ca^{2+}, PO_4^{3-} and pDNA concentrations were measured with appropriate assays.

RESULTS:p-CaP-NP matrices demonstrated osteoconductive properties through the absorption of Ca^{2+} and PO_4^{3-} ions, whilst p-MgP-NP matrices demonstrated a comparatively reduced mineralisation. Both monophasic matrices demonstrated a dose-dependent complexation of the pDNA payload to phosphate-salt nanoparticles. Absorption/release assays demonstrated that these properties are also reproduced in the biphasic matrices.

DISCUSSION & CONCLUSIONS:In-vitro absorption/release assays of anisotropic gradient mineralised hydrogels in a relevant SBF solution demonstrate desirable hydroxyapatite-like mineral precipitation in p-CaP-NP matrices but limited precipitation in p-MgP-NP matrices. This effect is substantiated in biphasic matrices, whereby a gradated precipitation of hydroxyapatite-like mineral is observed due to preferential precipitation in the p-Cap-NP phase. This anisotropic hydroxyapatite-like mineral precipitation effect in novel biphasic matrices in-vitro indicates their promising potential for OC tissue regeneration. Further development of the novel electrophoretic platform will allow manipulation of nanoparticle nucleation and growth mechanisms to modulate nanoparticle properties and optimise in-vitro transfection efficiencies.

Keywords: Hydrogels and injectable systems, Gene therapy

Fabrication of 3D Complex Shaped Fibrous Scaffolds with Enhanced Mechanical Strength for Meniscus Tissue Regeneration

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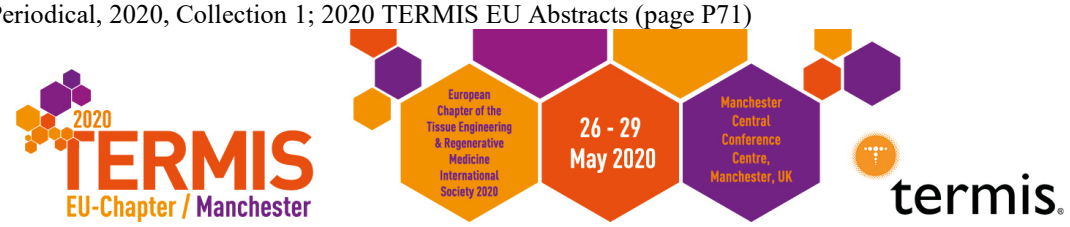
INTRODUCTION:Scaffolds are essential for cell support in tissue engineering. In our previous study, we have developed 3D fibrous scaffolds that can be fabricated in a variety of complex shapes. Interconnected fibrous networks was demonstrated to be particularly interesting as scaffolds due to a high porosity, interconnectivity and surface area. However, the weak physical properties of scaffolds were not suitable for meniscus regeneration. In this study, we developed a complex scaffold by introducing 3D printed supports into 3D fibrous scaffolds to enhance mechanical properties.

METHODS:Scaffolds were prepared by freeze drying method using polycaprolactone (PCL) 3D printed supports and short cut fibers such as poly(lactic-co-glycolic) acid (PLGA), and poly(lactic acid) (PLA). Highly viscous materials such as carboxymethyl cellulose (CMC) and alginate were used for uniform distribution of the fibers contained therein and for the various formation of complexed 3D shapes. Pore structure and morphology were observed by SEM, and potential of scaffolds for tissue engineering was evaluated by investigating the adhesion and proliferation of cells

RESULTS:Our scaffolds showed excellent 3D structure with inter-connecting pores and the cell adhesion rate of scaffolds reached more than 85%. Cell growth generally increases with time, and scaffolds containing 3D printing supports were superior to scaffolds without 3D printing supports in mechanical strength.

DISCUSSION & CONCLUSIONS:Complex shaped microfibrous 3D structure containing 3D printed supports has great potential as a biomimicking architecture for cell growth and maintaining cell functions.

Keywords: Biomaterials, Cartilage / joint and arthritic conditions



In situ gellable antimicrobial hyaluronan based hydrogels with tunable viscoelastic properties and rate of degradation

Martin PRAVDA, Lenka KOVAROVA, Evgeniy TOROPITSYN, Vladimir VELEBNY
Contipro a.s.

INTRODUCTION:Hyaluronan based in situ gellable hydrogels were suggested as suitable materials for the prevention of post-operative adhesions. The aim of this study was to develop an injectable hydrogel with antibacterial properties suitable for application by minimally invasive procedures. The hydrogel should cover surrounding tissues in order to prevent organ adhesions. The presence of antimicrobial agent should decrease the risk of post-operative infection.

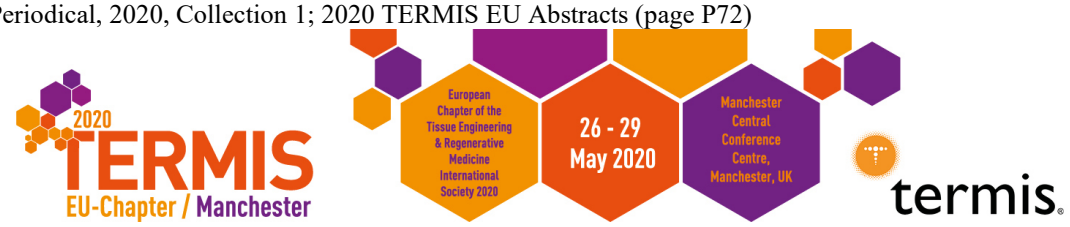
METHODS:Hyaluronan-tyramine conjugate (HA-Tyr) was synthesized according previously reported protocol [1]. The structure and molecular weight of the HA-Tyr conjugate were determined by 1H NMR and SEC-MALLS, respectively. Hydrogels were prepared by horseradish peroxidase (HRP) mediated crosslinking reaction [1]. Triclosan/hydroxypropyl-β-cyclodextrin inclusion complex (TCS/CD) was used as antimicrobial component. Gelation time and viscoelastic properties of hydrogel were evaluated by TA Instruments AR-G2 rheometer. Rate of hyaluronidase mediated degradation in vitro was measured by loss of mass in time. Bacterial strains Staphylococcus aureus, Escherichia coli, Clostridium sporogenes and yeast Candida albicans were used to evaluate hydrogel antimicrobial activity by diffusion plate method.

RESULTS:TCS is a hydrophobic drug poorly soluble in water. It is well known in the field of coating implantable devices. The TCS/CD inclusion complex was prepared to solubilize the drug in water in order to produce a homogenous distribution of TCS in the hydrogel. Hydrogels containing TCS in concentrations ranging from 0 to 1 mg/ml were prepared. Hydrogels containing at least 0,8 mg/ml of TCS exhibited sufficient antimicrobial efficacy towards all tested microorganisms.
Increasing concentration of TCS/CD complex decreased both gelation rate and elastic modulus of prepared hydrogel. This phenomenon could be caused by the formation of complex of the present cyclodextrin and tyramine moiety of HA-Tyr derivative. The existence of this complex could decrease the effectivity of HRP mediated tyramine oxidation and crosslinking process. Decrease of the crosslinking efficacy of hydrogel typically leads to higher rate of enzymatic degradation of such hydrogels. Surprisingly, hydrogels containing TCS/CD complex exhibited higher resistance to hyaluronidase mediated degradation in comparison with hydrogel with comparable viscoelastic properties but lacking the presence of TCS/CD.

DISCUSSION & CONCLUSIONS:HRP mediated crosslinking reaction of HA-Tyr derivative is well known process of preparation of hydrogel with tunable viscoelastic properties. Combination of this hydrogel with TCS/CD provides hydrogels with antimicrobial properties and enhanced resistance against the enzymatic biodegradation.

References:[1] Wolfova, L., et al., J Tissue Eng Regen Med, 2012. 6: p. 191.

Keywords: Drug delivery,



Model-based design of 3D-printed calcium-phosphate scaffolds for dental applications: from in silico design to in vivo validation

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INTRODUCTION:The use of ceramic biomaterials for dental bone regeneration or alveolar bone preservation is a reliable treatment option compared to autologous bone which involves higher morbidity. In this study, we report the development and use a computational model describing neotissue formation in 3D scaffolds. After calibration with in vitro experiments, we use the model to design 3D structures that in a calvarial model in the rat.

METHODS:A previously published model for neotissue growth in 3D printed Titanium scaffolds [1] forms the basis for this study. The level-set method is used to simulate the advancement of the neotissue inside the 3D scaffold. In a first step, curvature-based growth was tested in simple prismatic scaffolds that were manufactured using additive manufacturing in different calcium phosphate materials.
For the in vivo study, a lattice structure with square struts (called here orthogonal lattice) and a gyroid structure is selected. BioOss was taken along as a control. The scaffolds were implanted under a CaP shell on the calvaria of rats.

RESULTS:The in vitro studies confirmed the validity of using the curvature-based growth as a basic principle of our neotissue growth model. Using the model, we were able to run a large in silico screening experiment to determine optimal properties of our 3D scaffolds, ensuring both maximum neotissue growth and manufacturability. The in vivo results show bone formation in all tested cases. Whereas for BioOss and the orthogonal lattice, the amounts were rather limited, the amount of bone formed inside the gyroid structure was significantly higher, up to an average of 15%. Furthermore, the gyroid scaffold was the only one showing bone formation all the way at the top of the implant.

DISCUSSION & CONCLUSIONS:The computational model developed in this study was used to design geometries for 3D printed CaP-based scaffolds. In vivo testing of these scaffolds showed a strong improvement of the amount of bone formation in the model-based design (gyroid) over the clinical standard or a classical 3D printed geometry.

ACKNOWLEDGEMENTS:We gratefully acknowledge support from the Walloon Region (DGO6) through the BioWin project BIOPTOS (ID: 7560) and FNRS-FRFC (project ID: T.0256.16).

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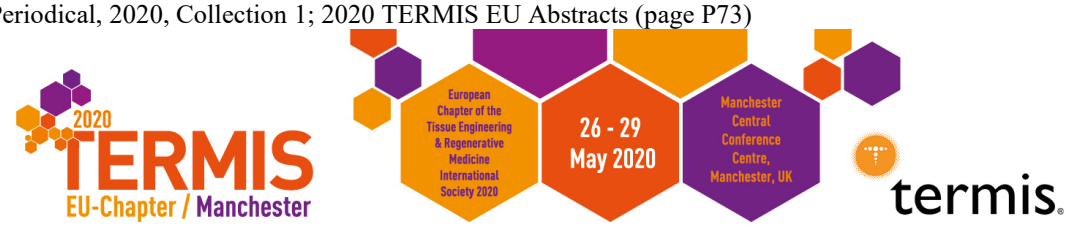
Keywords: Biomaterials, In silico models



Tissue Engineering the Oesophagus: Proof of Concept

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INTRODUCTION:Current treatments for oesophageal cancer and oesophageal atresia, that involves the repair of the entire thickness of the oesophagus, present various complications and challenges due to the lack of functional oesophageal replacement tissue. Through the combination of cells, scaffolds and biologically active molecules, tissue engineering presents an innovative approach to develop constructs that can mimic the multi-layered architecture of the oesophagus. This study utilises the combination of a novel biomaterial in the form of self-assembling peptide hydrogel, PeptiGel Alpha4 (Manchester BIOGEL), and a traditional biomaterial, polycaprolactone (PCL) scaffolds, to fabricate a proof of concept multi-layered construct to replicate the oesophageal tissue.

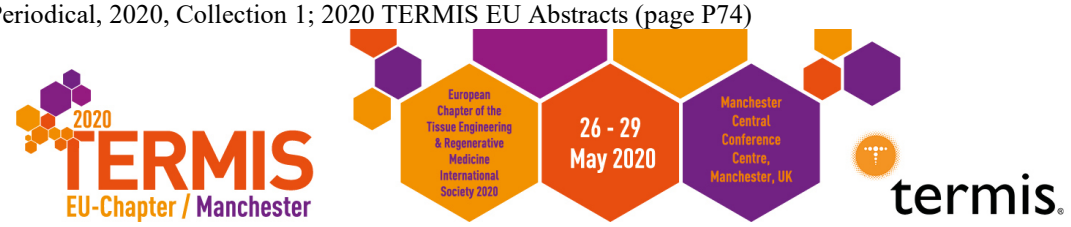
METHODS:Aligned PCL fibres were fabricated using a 15% PCL in chloroform solution and extruded at a flow rate of 1.5 ml/hr, with the application of 18.0 kV of positive voltage to the needle placed 20 cm from a grounded cylindrical collector that rotated at 1400 rpm.
Porcine oesophageal smooth muscle cells (pOSMCs) were seeded at 40,000 cells/cm² onto plasma-treated PCL scaffolds; and cell viability assay, alamarBlue metabolic assay and immunochemical analysis were performed at days 4 and 7.
3D culture of L929 connective tissue fibroblasts within PeptiGel Alpha4 was performed using a cell density of 100,000 cells/150 µL volume of hydrogel; and cell viability assay and immunohistochemical analysis were performed at days 7 and 14.

RESULTS:AlamarBlue metabolic assay recorded greater increases ($p<0.0001$) in the average fluorescence intensities on plasma-treated PCL scaffolds with pOSMCs compared to non-treated PCL scaffolds with pOSMCs at days 1, 4 and 7.
pOSMCs seeded onto plasma-treated PCL scaffolds also demonstrated positive staining by immunochemistry for the contractile phenotype marker protein alpha-smooth muscle actin at days 4 and 7.
Analysis of live/dead images showed that pOSMCs on non-treated PCL scaffolds at days 4 and 7 were rounded and not aligned, and pOSMCs on plasma-treated PCL scaffolds were elongated and aligned in the direction of the fibres.
Live/dead analysis of L929 fibroblasts cultured in 3D within PeptiGel Alpha4 indicated >90% cell viability at days 7 and 14.
Immunohistological analysis indicated type 1 collagen production by L929 fibroblasts cultured in PeptiGel Alpha4 at days 7 and 14.

DISCUSSION & CONCLUSIONS:The results collectively indicate that employing a combination of synthetic peptide hydrogels and aligned PCL fibres to tissue engineer the multi-layered structure of the oesophageal tissue is a viable option.

ACKNOWLEDGEMENTS:This research has been funded by UK Regenerative Medicine Platform (UKRMP2) and Manchester BIOGEL.

Keywords: Polymers - natural / synthetic / responsive, Hydrogels and injectable systems



Synthesis of Poly(3-Hydroxybutyrate-co-4-Hydroxybutyrate) [P(3HB-co-4HB)]-Bioactive Glass-Graphene Biocomposite for Translational Biomedical Applications

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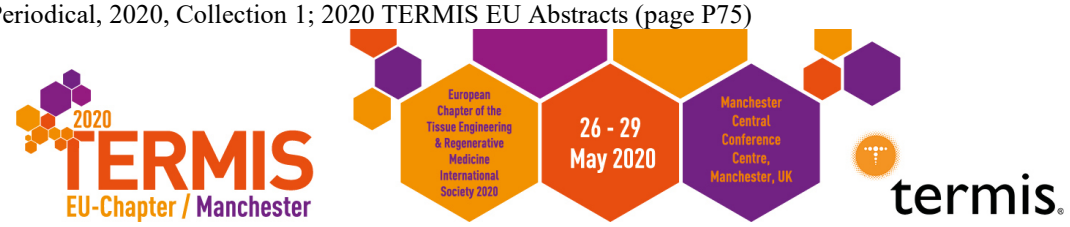
INTRODUCTION:Over the years, development of novel tissue engineered biomaterials for wound repair and regeneration has identified key parameters required to successfully translate them to biomedical applications. Polyhydroxyalkanoates (PHAs) are bio-based microbial thermoplastic polyesters of which their monomers are recognised as natural metabolites found in humans and other mammals. PHAs exhibited essential biocompatibility and biodegradability properties and has high potential as versatile wound dressing for topical application. However, a key disadvantage of PHAs lies in their hydrophobic nature which, ultimately, limits additional exploitation of the material. As such, this study aims to further optimise and enhance PHAs by using surface modifiers such as bioactive glass (BG) and graphene to increase the mechanical, physical and biological characteristics of the biocomposite.

METHODS:P(3HB-co-4HB) copolymer, isolated from Cupriavidus sp. USM1020 was biosynthesised via single stage flask (50mL/200rpm) and bioreactor (3L/300rpm) inoculation supplemented with 1,4-butanediol and 1,6-hexanediol carbon sources. Copolymer formed via both methods were analysed and compared in terms of chemical (monomer compositions), physical and mechanical characteristics using GC, FT-IR, DMA and DSC. Sol-gel BG and liquid-exfoliated graphene (fixed concentration of 0.25, 0.5, 1, 2 and 4% w/v for both) were added to the matrix and casted via solvent casting technique. The chemical, physical and mechanical characteristics of the novel composites were subsequently assessed via GC, DMA, SEM and water contact angle whereas biological/biocompatibility was assessed using the human (melanoma) fibroblast cell line (Malme-3/3M) and the alamarBlue™ (AB) assay.

RESULTS:P(3HB-co-4HB) copolymers were successfully isolated and characterised, where inoculation via bioreactor produced higher percentage of 4HB yielded at ±90 mol% and PHA recovery of ±80 wt% as compared to stage flask. Higher 4HB% matrix illustrated higher elongation at break and tensile strength (TS) of ±260% and ±22 MPa respectively as molecular weight within the backbone chain increased. Incorporation of BG and graphene resulted in slight decrease of TS of ±20 MPa, supporting the potentiality of this formulation as topical/dermal biocomposite. Wettability improved as concentration of the formulation increased, whilst AB assay demonstrated enhanced human cell attachment, viability and proliferation.

DISCUSSION & CONCLUSIONS:Surface modification of high 4HB% P(3HB-co-4HB) copolymer through the recruitment of BG and graphene improves and enhances its physical strength, hydrophilicity and biocompatibility. Taken together, these initial findings demonstrate the feasibility of the novel PHA biocomposite as a possible topical/dermal wound dressing and, further optimisation of the material could lead to other potential applications such as patches for anti-cancer treatment and diagnostic substrates.

Keywords: Composite materials, Skin



Optimising the mechanical stimulation regime to enhance collagen production from 3D cultures of human dermal fibroblasts

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INTRODUCTION:Biologically derived scaffolds are successfully used in tissue engineering as they are biocompatible and can be easily remodelled in vivo, however using decellularized tissue can result in problems with scaffold reproducibility and risk of potential disease transfer. One solution is to create a reproducible, cell-derived biological scaffold in vitro by culturing cells on a biodegradable, synthetic scaffold which is exposed to mechanical stimulation. The cellular component is then removed before using. This method has been employed to create tissue engineered blood vessels[1], but there is limited information available on the optimisation of this process. This project therefore aims to develop this process to create a mechanically enhanced and architecturally organised extracellular matrix (ECM)-based cell-derived scaffold through mechanical stimulation of cells growing on a synthetic scaffold in vitro.

METHODS:We used polyglycerol(sebacate)-methacrylate (PGS-M) polyHIPE as the synthetic scaffold as it is biocompatible and biodegradable[2]. It also allows us to control and design the complex structure and mechanical properties during the fabrication process, generating a porous structure within which cells can grow and produce ECM.
Human dermal fibroblasts (HDF) were seeded onto 50% methacrylated PGS-M scaffolds, cultured for 1 week and exposed to a range of mechanical stimulation profiles using Ebers TC-3 bioreactor. After 1 week in culture MTT and Sirius red assays were used to determine cell metabolic activity and total collagen production for each scaffold.

RESULTS:Our results show that application of intermittent pulsatile strain significantly increased cell metabolic activity compared to both static cultures and scaffolds under constant pulsatile strain, while incrementally increasing the strain applied to the scaffold enhanced collagen production.

DISCUSSION & CONCLUSIONS:Mechanical stimulation has been shown that it can induce cell proliferation and length of tissue in vivo[3], this study demonstrates mechanical strain can influence cell proliferation and collagen production for cells in vitro, while revealing that optimisation of mechanical stimulation regime is necessary.
We have therefore successfully created the first prototype of a robotic bioreactor with force sensing, distance sensing and real-time stiffness measurement to apply mechanical strain and monitor the changing scaffold during culture period. This will allow us to more readily understand the effects of mechanical stimulation and control the force according to the condition of the samples as the synthetic scaffold degrades and ECM is generated.
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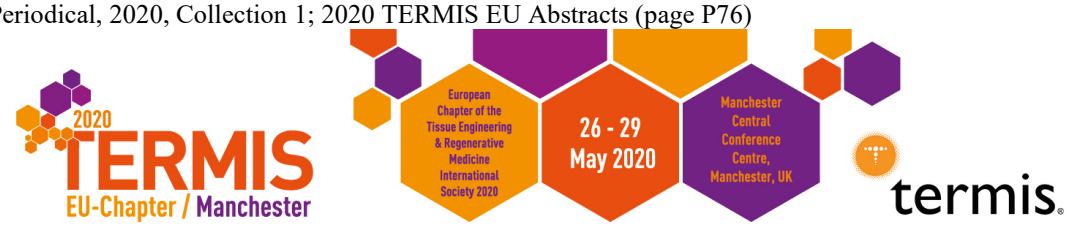
Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Bioreactors



Development of GelMA/GrowDex® hybrid hydrogels for biomedical applications

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INTRODUCTION:Due to their tuneable mechanical properties and biocompatibility characteristics, novel hydrogels can be developed and/or enhanced to suit an extensive range of biomedical applications such as cell and drug delivery, wound healing, ophthalmic research and tissue regeneration. GelMA (gelatin-methacry(late)/(loyl)) is a semi-synthetic hydrogel that exploits the biological signals inherent in the gelatin molecule, while allowing control of mechanical properties, has proved itself as one of the most versatile hydrogels available for 3D cell culture and bioprinting (1). GrowDex® is a cellulose derived animal-free, ready to use hydrogel that mimics the extracellular matrix (ECM) and supports cell growth and differentiation with consistent results. As such, this study focuses on the development and optimisation of novel GelMA/GrowDex® hybrid hydrogel formulations, which may provide synergistic benefits in terms of their mechanical, physicochemical, and biological characteristics.

METHODS:GrowDex® (UPM Biomedical, Finland), (in-house) synthesised GelMA from gelatin (porcine, ~300 g Bloom, Type A, Sigma-Aldrich, Poole, UK) and combinations of the above were assessed using a range of techniques including dynamic mechanical analysis (DMA), SEM, swelling degree, ATR-FTIR and THz sensing. Drug diffusion characteristics were assessed using fluorescein isothiocyanate (FITC) and Franz cells. For biocompatibility assessments, C6 glial (ATCC® CCL-107™)), L929 fibroblast (ATCC® CCL-1™) and human corneal cells were seeded into the hydrogels at 1x105 cells/mL for 1, 3, 5 and 7 days. Assessment of metabolic activity and viability were measured using the Alamar Blue and Live/Dead and LDH assays, respectively.

RESULTS:GelMA and GrowDex®, alone, demonstrated suitable characteristics as a biomaterial and cell carrier/storage medium. A number of novel hybrid hydrogels were successfully and reproducibly fabricated. Key differences in mechanical characteristics of these gels such as viscoelasticity, storage and loss moduli, stress relaxation behaviour, and pore size were identified and shown to be related to the specific GelMA/GrowDex® ratio. Enhancement of the biological and biopharmaceutical behaviour of the hybrid materials was also identified compared to the native gels.

DISCUSSION & CONCLUSIONS:As such, further optimisation of these “new” hybrid gels could be tailored to suit a specific endpoint biomedical application.

ACKNOWLEDGEMENTS:Financial support for ZKE has been received from Turkish Ministry of National Education. Aspects of this work has also been kindly funded by UPM - Kymmene Corporation (Finland).

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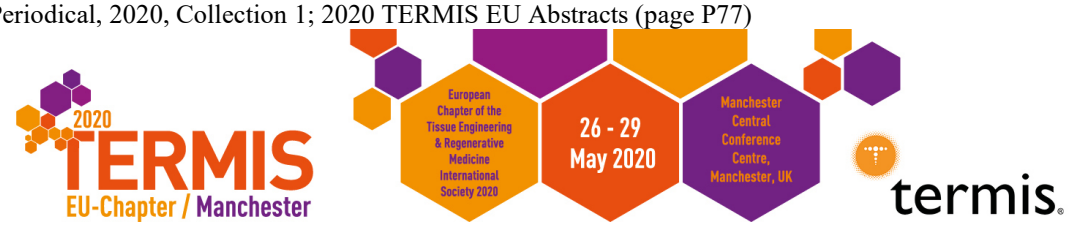
Keywords: Biomaterials, Cell therapy



The in vitro and in vivo characterization of decellularized chicken eggshell membrane for translational regenerative applications

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INTRODUCTION:The chicken eggshell membrane (ESM) is an exceptional biomaterial in nature: its composition includes a wide range of bioactive components and also demonstrates unique biocompatibility/ biodegradability characteristics that can be exploited as a potential wound healing agent in the form of a dressing for either dermal or ophthalmological applications [1]. The ESM composes of three distinct layers: the inner and the outer membranes (fibrous structures) and the limiting membrane (dense structures). The ability to separate the ESM from the eggshell is vital to its application as a biomaterial and various methods of extraction of ESM have been previously employed which lead to differences in their final composition, structure, biological characteristics and wound healing effects [2, 3]. In this study, ESMs were extracted using a number of different techniques and thereafter characterized in terms of their physical and biological properties.

METHODS:ESM samples were prepared using either a manual, an optimised acid- and/or chelator-based decellularization protocol. The physical and mechanical characteristics of the ESM were assessed using standard material testing techniques including DMA, SEM, WCA, FT-IR and THz sensing. In vitro biocompatibility, cytotoxicity, cell attachment and spreading assays were performed using human (melanoma) fibroblast cell lines (Malme-3/Malme-3M), immortalised human corneal epithelial cells (ihCEC) and cornea mesenchymal (stromal) stem cells (cMSC). The chicken embryo chorioallantoic membrane (CAM) assay was employed to ascertain the angiogenic potential of the extracted membranes. In vivo evaluation of the healing properties was assessed using standard wound models in mice and rats for skin and eye applications, respectively.

RESULTS:Complete ESM samples were successfully isolated and prepared from native materials and fully characterized. Malme-3M, ihCEC and cMSC cells cultured on the extracted ESM samples demonstrated high biocompatibility in terms of high cell attachment, spreading, viability and proliferation rates/characteristics and also allowed the promotion of new blood vessels (i.e. pro-angiogenic) in the CAM assay. In vivo studies demonstrated pro-healing characteristics with no local adverse effects.

DISCUSSION & CONCLUSIONS:This work summarizes the development of an ESM-based wound dressing: modulation of the physical and biological characteristics of the membrane can be achieved via the isolation/extraction/preparation technique used and, as such, the novel biomaterial can be adapted to suit its final translational application i.e. skin and/or eye.

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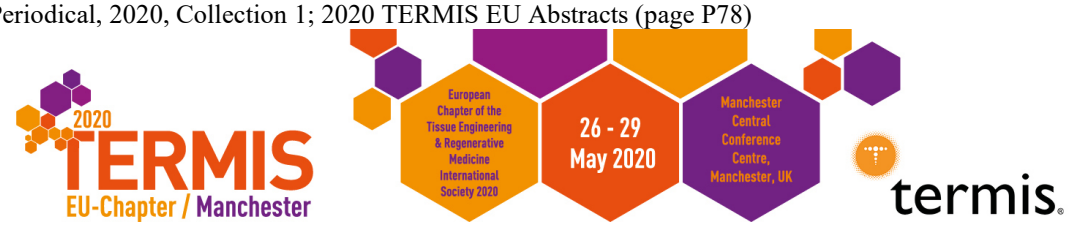
Keywords: Biomaterials, Decellularised matrices



In vitro characterization of ion-doped silicate microparticles for scaffold-based endochondral bone regeneration

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INTRODUCTION:Healing of large bone defects often results in the formation of non-unions and remains an unsolved clinical challenge [1]. Inducing endochondral ossification (EO) is a promising approach to heal large bone defects via an intermediate cartilage phase [2]. Recently, we introduced a cell-free soft collagen scaffold with a channel-like pore architecture inducing EO for healing of large bone defects in rats [3]. Here, we aim for an additional incorporation of ion-releasing silicate microparticles to enhance the endochondral healing potential of the scaffold. To evaluate their biocompatibility, silicate microparticles were tested in an in vitro approach.

METHODS:Lithium (Li)-, magnesium (Mg)-, strontium (Sr)- or zinc (Zn)-doped silicate microparticles were produced by INNOTERE GmbH applying sol-gel technology. Incorporation into the collagen scaffold walls (1.5% wt/wt) was carried out by Matricel GmbH. Before investigating potential positive effects on EO, the biocompatibility of silicate particles alone and incorporated into collagen scaffolds was evaluated. Human bone marrow mesenchymal stromal cells (hBM-MSCs) were seeded on transwell membranes and cultivated in expansion medium conditioned with ion-doped silicate particles. Cell density and morphology were evaluated using nuclei and β-Actin immunohistological stainings and confocal microscopy. Furthermore, hBM-MSCs were seeded in culture plates and ion-doped silicate particle-collagen scaffolds were added into transwell insert. Biocompatibility was assessed by quantifying metabolic activity and DNA content.

RESULTS:Zn-doped silicate microparticles revealed toxic effects on hBM-MSCs, when not incorporated in collagen scaffolds. For all other microparticle types and all silicate particle-collagen scaffolds continuous cell proliferation was observed. However, differences in cell proliferation and cell morphology were detected between the groups indicating potential effects of ions released from silicate microparticles on cell function.

DISCUSSION & CONCLUSIONS:Biocompatibility of tested microparticles and scaffolds was confirmed, except for non-incorporated Zn-doped silicate microparticles. Additional processing steps, performed during the incorporation of silicate microparticles into the scaffold, might alter the ion release behaviour and therefore particle and scaffold bioactivity, respectively. This would explain the opposing biocompatibility results for Zn-doped silicate microparticles. However, further investigations are required to understand this process. A potential enhancement of endochondral healing by incorporation of ion-doped silicate particles is currently evaluated by in vitro chondrogenesis and osteogenesis of hBM-MSCs within particle-loaded scaffolds.

ACKNOWLEDGEMENTS:This work has been funded by the Federal Ministry of Education and Research (Grant no. 13XP5048D, 13XP5048B, 13XP5048A).

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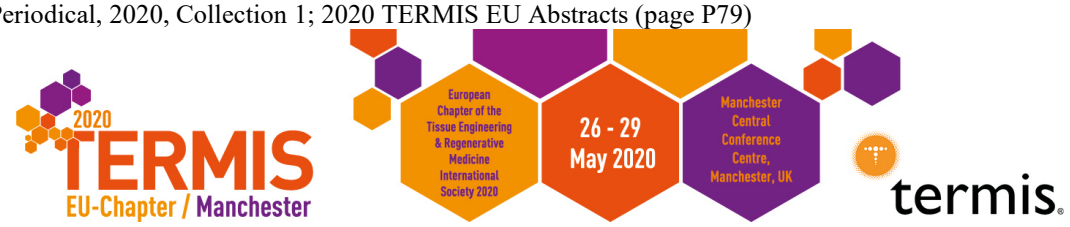
Keywords: Bone and bone disorders (osteoporosis etc),



Development of Tissue-Like Substitutes Based on Different Types of Agaroses. A Biomechanical and Biocompatibility Study

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INTRODUCTION:Agarose biomaterials have been previously used as scaffolds for the generation of several types of human tissue substitutes by tissue engineering (1), including the human skin, palate, oral mucosa, cornea, cartilage and nerve. The recent development of different types of agaroses with different biological and mechanical properties open the door to novel developments specific for each tissue type. In the present work, we have generated different agarose-based tissue-like substitutes and we have carried out a biomechanical analysis to determine which biomaterials could be more suitable for specific tissue engineering applications.

METHODS:Human tissue-like substitutes were generated by culturing human primary cell cultures in scaffolds based on 5 different types of agaroses (LM, D2LE, D1LE, MS8 and D5) previously developed by Hispanagar, SA. Each agarose type was solved in PBS at a final concentration of 0.3, 0.5, 1 and 3%. Then, the biomechanical properties of each biomaterial were analyzed after 24h of culture using an Instron biomechanical analyzer. Biocompatibility was assessed by quantifying free DNA released from the cells to the culture medium after the same follow-up period. As positive controls, living cells cultured in 2D systems were used, whereas cells treated with triton X-100 were used as negative controls.

RESULTS:Our biomechanical analysis revealed that the biomechanical behavior of each type of scaffold was statistically related to the concentration of agarose, as the concentration correlated positively with the Young modulus ($p<0.05$) and the stress at fracture ($p<0.05$). In addition, we found a statistically significant correlation between the strain at fracture and the agarose type ($p<0.05$), with the highest values of strain at fracture found for D1LE and D5. Regarding DNA release, all tissue-like substitutes showed very low amount of free DNA, being similar to positive controls and significantly lower than negative controls ($p<0.05$).

DISCUSSION & CONCLUSIONS:These results demonstrate that all tested agarose types are highly biocompatible, but their biomechanical properties are directly related to the agarose type and concentration. We therefore conclude that a specific agarose type and concentration should be selected for the development of bioartificial tissues related to specific environmental forces and biological functions.

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Keywords: Biomaterials, Skin

Biocompatibility of Fibrin-Agarose Hydrogels Crosslinked with 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide in Combination with N-Hydroxysuccinimide for Tissue Engineering

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INTRODUCTION:Chemical crosslinkers and novel biofabrication methods such as nanostructuration allow the generation of biomaterials with improved biomechanical properties such as stiffness and elasticity able to support the stress of the native tissues. However, biocompatibility of tissues subjected to these procedures may be impaired (1). The aim of this study is to evaluate the biocompatibility of non-nanostructured and nanostructured fibrin-agarose hydrogels (FAH) crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide with N-hydroxysuccinimide (EDC:NHS).

METHODS:FAH were generated from human plasma and subjected to chemical crosslinking with EDC:NHS at a concentration of [2:1] and [4:1]. Then, half of the hydrogels were nanostructured by applying plastic compression methods (2). Primary cultures of human dermis fibroblasts were cultured on the surface of each type of biomaterial. Histological analyses were performed by scanning electron microscopy (SEM) in order to identify the morphostructural pattern of each type of biomaterial and to analyze cell attachment and cell morphology. Furthermore, Live/Dead cell viability and WST-1 cell proliferation assays were used to study ex vivo biocompatibility. Statistical analyses were carried out using Mann-Whitney's U and Kendall correlation tests.

RESULTS:SEM analysis showed that the typical fibrillary pattern of FAH was not altered after improving the biomechanical properties of these biomaterials by using EDC:NHS crosslinking. However, crosslinking was able to reduce porosity, and the size of the interfibrillar pores was significantly lower in crosslinked FAH as compared to non-crosslinked FAH, with a significant correlation with the concentration of EDC:NHS in both nanostructured and non-nanostructured FAH. Ex vivo Live/Dead and WST-1 cytotoxicity analyses revealed high cell viability and functionally in all biomaterials subjected to cross-linking, suggesting that the process did not modify cell viability.

DISCUSSION & CONCLUSIONS:Improvement of the biomechanical properties of FAH by using EDC:NHS crosslinking agents was associated to a change in the microporous pattern of the biomaterials but did not alter the excellent biocompatibility of FAH. In conclusion, EDC:NHS could be used for the generation of bioartificial tissues with high biomechanical requirements such as the human skin, nerve, cornea, palate, cartilage or oral mucosa.

ACKNOWLEDGEMENTS:Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) from the Spanish Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grants FIS PI17/0391 and PI17/0393, PI18/331 and PI18/332 (co-financed by ERDF-FEDER, European Union) and Consejería de Salud y Familias, Junta de Andalucía, Spain, grant CS PI-0257-2017.

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Keywords: Biomaterials,

Biodegradation of Fibrin-based Biomaterials Used in Tissue Engineering. A Time-Course in vivo Analysis

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INTRODUCTION:Natural biomaterials such as fibrin should able to mimic the structure and composition of the human native extracellular matrix with none or minimal host inflammatory response. These biomaterials should be biointegrated in a reasonable period while supporting cell viability and function (1,2). In general, fibrin-based biomaterials are expected to show proper biointegration and biosafety levels, but the molecular changes and the definite histological changes driven by these biomaterials are not fully understood. The aim of this study was to perform a time-course assessment of in vivo biocompatibility of fibrin-based hydrogels at the histological, histochemical and immunohistochemistry levels in rats.

METHODS:Fibrin-based hydrogels were generated and subcutaneously implanted in 25 male Wistar rats and evaluated after 1, 3, 5, 9 and 12 weeks (n=5 each). Five healthy animals were used as controls. In each period, animals were subjected to histological, histochemical and immunohistochemistry studies of the grafted biomaterials and vital organs (liver, kidney, spleen and regional lymph nodes).

RESULTS:Histology revealed an initial and localized inflammatory response around the implanted biomaterials followed by a progressive invasion of host cell, mainly macrophages, involved in biomaterial biodegradation. In addition, an intense process of extracellular matrix remodeling and neovascularization was found from the second week, with the sequential synthesis of collagen and other extracellular matrix molecules. Grafted biomaterials were successfully biodegraded after 12 weeks without any associated histopathological alteration in the implanted zone or distal vital organs.

DISCUSSION & CONCLUSIONS:Our results showed a progressive and active biodegradation process mediated by macrophages without any local affection and no impact on the structure and function of distal organs was observed. These preclinical results indicate that fibrin-agarose hydrogels are highly biocompatible with an acceptable biodegradation rate. Furthermore, at the implantation zone these hydrogels supported a progressive neovascularization process and extracellular matrix remodeling. This study suggest that fibrin-agarose-based hydrogels could be a safe and an useful alternative for tissue engineering applications.

ACKNOWLEDGEMENTS:Supported by Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) from Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grants FIS PI17/0391, PI17/0393, PI18/0331 and PI18/0332 (co-financed by ERDF-FEDER, European Union) and by Consejería de Salud y Familias, Junta de Andalucía, Spain, grants PI-0257-2017, PE-0395-2019 and PI-0442-2019.

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Keywords: Biomaterials, In vivo and animal models

Topographical guidance of hierarchical mineralization

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INTRODUCTION:Enamel is well known as the hardest tissue in the human body1, composing of aligned hydroxyapatite (HAp) crystals arranged along the c-axis, and the behaviour of apatite crystal growth can be guided by self-assembly, forming amorphous precursors with different morphologies as templates.2 The function of enamel relies on its structure, and its structure on the organic-inorganic interaction and the deposition of precursors within the biomolecule media.2 Given this potential, a variety of mineralizing material platforms are emerging.3 We have recently reported on the possibility to modulate the interplay between order and disorder of an elastin-like protein (ELP) to nucleate and trigger the growth apatite nanocrystals in a hierarchical manner.4 However, this mineralization process does not provide spatial control of the mineralization process. The capacity to direct the anisotropic growth of such structures would have important implications in the design of materials to repair or regenerate enamel. Here, we investigate the use of precise surface topographies to spatially control the growth of apatite nanocrystals (Fig. 1).

METHODS:Soft lithography techniques were used to fabricate ELR membranes comprising precise micro topographies while maintaining the crucial ELP order-disorder synergy.

RESULTS:The results demonstrate that the growth of the hierarchical structures can be guided through microchannels of specific dimensions. Using a wide variety of analytical techniques including Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) spectroscopy, Focused Ion Beam SEM (FIB-SEM), Transmission Electron Microscopy (TEM) and Selected Area Electron Diffraction (SAED), we identify and describe key mechanistic features of this topographical effect and attempt to outline rules for the design of hierarchically mineralized materials more broadly.

DISCUSSION & CONCLUSIONS:We have attempted guide the growth of mineralized structures via an ion-diffusion nucleation pathway. Overall, the study explores the effect of surface topographies on guiding the growth of HAp hierarchical structures spatially. This approach successfully guides the growth of HAp hierarchical structures, which provides a new way of fabricating complex mineralised structures.Given the strong structure-function relation observed in mineralized tissues and biological materials, material platforms that can control the directionality of mineralization and enable the growth of hierarchical mineralized structures would have tremendous opportunities in materials science. In addition, further understanding of underlying mechanisms of mineralization would have implications in a broad range of fields expanding from medicine to engineering.

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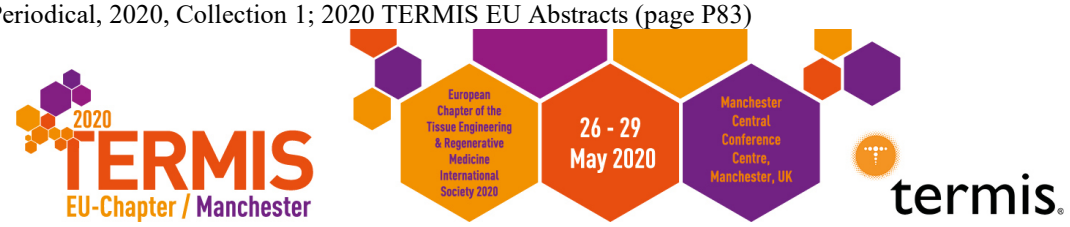
REFERENCES:N/A

Keywords: Bone and bone disorders (osteoporosis etc), Other



Engineering the Liver Using Self-assembled Peptide Hydrogels

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INTRODUCTION:Liver diseases are becoming a significant public medical burden[1]. To overcome the lack of donor livers and high cost of therapy, this project aims to exploit a long-term culture system that maintains hepatocyte specific functions[2] and cell viability for liver regeneration using self-assembled peptide hydrogels to mimic the normal hepatic extracellular matrix (ECM). Previous work[3] proved that a three dimensional cell culture environment could keep differentiated hepatocytes compared with in vitro cell culture on a flat surface.

METHODS:All tested self-assembled peptide hydrogels (SAPHs), PeptiGel® Alpha1, AlphaX, DeltaX and FE, were purchased from Manchester BIOGEL. HepG2 cells (1x10^{6}cells/ml) were suspended in 166µl fresh media then encapsulated within 1ml SAPHs to get a homogeneous cell-gel mixture. The gels were placed into inserts with media. Cell viability, proliferation and albumin secretion were investigated. The stiffness of peptide hydrogels compared to porcine liver tissue were measured.

RESULTS:The theoretical net charges of peptide hydrogels were calculated. For mechanical stiffness of hydrogels, at 1 Hz, the G' of Alpha1 was 6.00 ± 0.10 kPa, AlphaX1 was 8.56 ± 0.25 kPa, AlphaX2 was 16.88 ± 0.79 kPa, DeltaX1 was 1.39 ± 0.17 kPa, DeltaX2 was 2.19 ± 0.11 kPa, FE was 0.93 ± 0.16 kPa. The porcine liver tissue sample was 64.49 ± 11.84 kPa. The cell viability assays showed that over 14 days in vitro culture, HepG2 cells proliferated within Alpha1 and DeltaX. ELISA assay displayed a significant increasing trend of albumin production for cells grown in Alpha1 during 7 days culture.

DISCUSSION & CONCLUSIONS:Hepatocyte viability could be maintained within SAPHs during 3D in vitro culture. The increasing level of albumin production over 7 days suggested HepG2 cells could maintain hepatic functions within SAPHs. Results suggested a suitable SAPHs stiffness was between 1~6 kPa in media and that the net charge of SAPHs could effect hepatocyte survival and proliferation.

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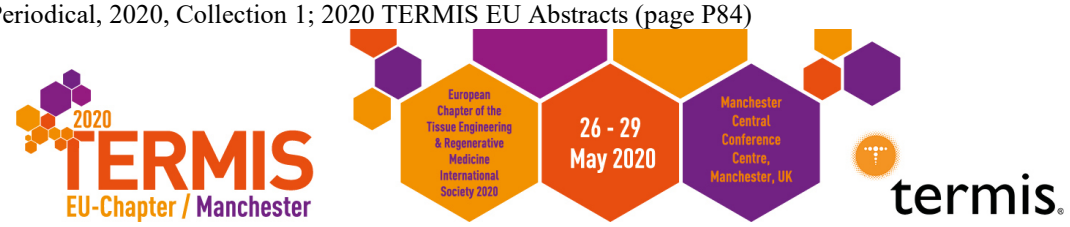
Keywords: Biomaterials, Hydrogels and injectable systems



Enhancement of electrospun polycaprolactone scaffold biocompatibility

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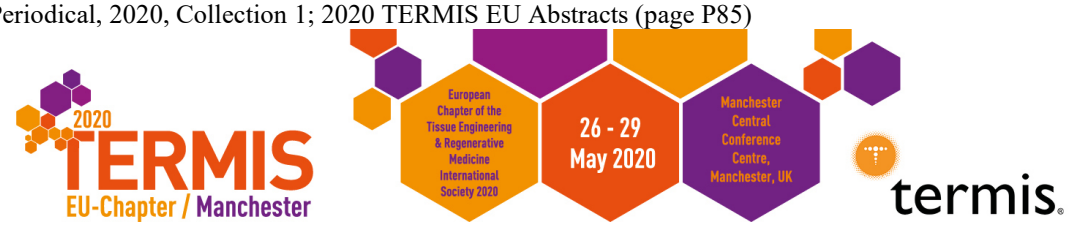
INTRODUCTION:Synthetic and natural polymers are used to fabricate scaffolds for tissue engineering. Synthetic scaffolds offer several advantages compared to scaffolds made from natural polymers, including longer shelf-life, cost efficacy, and limited risk of rejection. Polycaprolactone is one of the most suitable and desired synthetic biodegradable polymers having strong mechanical properties and biocompatibility. Although polycaprolactone has properties that are highly attractive for tissue engineering, the problem related with its hydrophobicity remains. In case of replicating the structure and properties of natural human tissues, electrospinning is one of the most popular method to manufacture the scaffolds. In order to adapt the polymeric fibre for tissue engineering, it is crucial to improve the hydrophilicity of the polymer and thus to create a suitable growth medium for cells.

METHODS:The aim of this study was to fabricate polycaprolactone (PCL) construct with uniform morphology using solution electrospinning, analyse the O_3 in water treatment effect on PCL scaffold for cell growth and proliferation. Sample fibres (weigh 20 to 50 mg) were ozonated for the period of 30 to 120 minutes. Scaffold physicochemical properties and biocompatibility were measured using ATR-FTIR, XRD, water contact angle, number of carboxyl groups determination, hydrophilic analysis, mechanical analysis and cell proliferation evaluated by the CCK-8 assay.

RESULTS:The ozone treatment resulted in formation of O-H group in the main chain of PCL scaffolds and improved the hydrophilicity. The duration of treatment had a positive effect on cell proliferation. After one day cell count of the longest O_3 duration treatment time was approximately 4 times greater compared to untreated scaffold. Although the cell count dropped substantially after 3 days (from 1.74 ± 0.02 to 1.08 ± 0.35), the remaining cells remained viable and proliferated for up to 14 days (0.49 ± 0.03).

DISCUSSION & CONCLUSIONS:The O3 treatment of electrospun polycaprolactone scaffold demonstrated an improve on performance of cell growth, with potential applications in tissue engineering.

Keywords: Fibre technology, Polymers - natural / synthetic / responsive



Mechanical stimulation of dental pulp stem cells for fibrocartilage tissue engineering

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INTRODUCTION:During development, neural crest cells give rise to facial structures including the mandibular condyle (Kruijt Spanjer et al. 2017, Stocum et al. 2018). Several neural crest-derived stem cell populations have been identified, such as dental pulp stem cells (DPSCs) (Gronthos et al. 2000). Studies have indicated that mechanical loading influences proliferation, migration, and maturation of chondrocytes in the mandibular condyle during development (Kantomaa et al. 1994, Sobue et al. 2011, Utreja et al. 2016). The aim of this study is to evaluate the effects of mechanical loading of DPSCs in the context of tissue engineering of fibrocartilage for mandibular condyle regeneration.

METHODS:DPSCs were isolated from the dental pulp of 3 healthy patients (22±5Y), expanded until passage 4, and encapsulated in 7% gelatin-methacryloyl (GelMA) hydrogel discs (80% degree of functionalization, d=8mm, h=4mm) at $20*10^6$ cells/mL using a visible light crosslinking system (Lim et al. 2019). One day after encapsulation, 4 discs/donor were harvested, while the rest were mechanically loaded using a custom multiaxial bioreactor (Li et al. 2010) and subjected to ±27° shear at 1Hz and 10% compression. Loading was applied for 1h/day for 5 consecutive days for a total of 4 weeks in the presence or absence of 10 ng/mL TGF-β1. Non-loaded discs were used as controls. After 4 weeks, gene expression levels of SRY-box 9 (SOX9), collagen type 1 (COL1A1), COL2A1, cartilage oligomeric matrix protein (COMP), aggrecan (ACAN), and lubricin (PGR4) were measured in half of the samples, while the other half was stained with a safranin-O/fast green staining to evaluate matrix maturation.

RESULTS:Expression of SOX9, COL1A1, COMP, and PGR4 was upregulated after 4 weeks culture with or without TGF-β1. In all samples COL2A1 and ACAN were not detectable. No differences were seen between the loaded samples and the non-loaded controls, regardless of exogenous TGF-β1 supplementation. Histological evaluation revealed no beneficial effect on matrix maturation in the loaded samples compared to the non-loaded controls. Additional supplementation of TGF-β1 did not improve matrix deposition.

DISCUSSION & CONCLUSIONS:Mechanical loading during differentiation of DPSCs does not increase the expression of chondrogenic genes as well as extracellular matrix deposition. Unlike the supplementation of exogenous TGF-β1, mechanical loading does not seem to be pivotal for in vitro DPSC differentiation
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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Differentiation

Biomechanical characterization of engineered tissues and implants for tissue engineering applications

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INTRODUCTION:Biomaterials along with engineered tissues and implants are essential part of any operations for tissue repair and regenerations. Besides chemical, mechanical and rheology properties of templates and engineered products are very important - unfortunately, many different methods of biomechanical testing give rather different outcomes so it is not easy to obtain realistic, true properties.
Biomechanical properties are also critical to consider a proper cell culture system, and they need to be evaluated in a correct and physiologically relevant way. When cell culture systems become more specific, there are also more scientific, technical, and regulative demands to quantify the properties.

METHODS:In this work we analyse the biomechanical properties and behaviour of scaffolds and hydrogels aimed at proper mechano-transduction for applications in articular cartilage and laryngeal tissue repair. We compare traditional approach of measurements with new idempotent analysis which does not impose prior model type for a material and incorporates material loading history.

RESULTS:The data obtained for polymeric porous scaffolds and hyaluronic based hydrogels show additional data which can be extracted form rather simple measurement protocols which were designed to be closer to physiological conditions. These invariant data can be used for predictive estimations of biomaterial behaviour as they will be not time- or frequency dependent.

DISCUSSION & CONCLUSIONS:It is now anticipated that the boundaries between biomaterials development, biomaterials and tissue biomechanics are getting lower and there is much more understanding in role of biomechanical cues in biomaterials applications. For the purpose of the better integration, we suggest a definition of biomechanology, which might combine practical, controllable and measurable biomechanical parameters on the level of tissues and organs, with a closer physiological relevancy. It would for example be focused on how mechanical stimulation would modulate tissue regeneration and biomaterial-tissue interaction, comprising essential parts of practical biomechanics, mechano-biology, mechano-immunology and perhaps mechano-bacteriology (to add effects of pathogens and formation of biofilms) complemented by proper and correct biomechanical testing.
One example could be in 3D printed and conventional materials, which were found to exhibit significant differences in biomechanical properties not originally expected. Such differences have to be quantified and taken into account already at the design stage as the new solution might generate different biomechanical environments at the same anatomical location, potentially leading to undesired outcomes.

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References:https://www.panbiora.eu/

Keywords: Biomaterials, Enabling technologies

Nanogrooved microdiscs modulate response of co-cultured stromal and endothelial cells within liquefied-core capsules in dynamic conditions

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INTRODUCTION:The influence of surface topography on cell behaviour and differentiation ability of stromal cells has been established in 2D. Yet, the role of topography in a tissue mimetic 3D-environment is poorly explored. We recently proposed nanogrooved microdiscs (topodiscs) for a bottom-up cell-mediated 3D fabrication, which led to the osteogenic differentiation of adipose-derived stromal cells (ASCs) without supplemental osteoinductive factors.[1] Combining topodiscs with the liquefied-core capsules technology,[2] we aim to perform a sequential seeding of 3D-preaggregated topodiscs and ASCs with endothelial cells (ECs). Upon mild core liquefaction and dynamic culturing ASCs and ECs will crosstalk directly. While topodiscs provide topographical cues to direct osteogenic differentiation of pre-adhered ASCs, the resultant 3D-aggregates provide living domains for intercellular communication with ECs. Our hypothesis is that by combining topographical cues with cell signalling pathways, prevascularised bone-like microtissues can be developed in an autonomous fashion.

METHODS:Topodiscs were produced via nanoimprinting of spherical polycaprolactone microparticles between water-soluble polyvinyl alcohol counter-moulds of nanogrooved templates. Uniform ASC-topodisc microaggregates were produced using Aggrewell400 well-plates. Then, microaggregates and ECs were encapsulated within liquefied-core capsules, and upon mild liquefaction cells crosstalk directly. Spherical microparticles lacking topographical cues were used as control.

RESULTS:Topodisc production via nanoimprinting led to a homogeneously nanogrooved surface. After 24h, uniform ASC-topodisc microaggregates were formed. ASC and EC labelled with lipophilic fluorescent dyes allowed to assess relative cell positioning within the capsule core. The successful biological activity was confirmed via increased DNA content and merging of several microaggregates into macro 3D constructs. At 21 days post-encapsulation with topodiscs, cells differentiated towards the osteogenic lineage in static conditions for both mono and co-cultures as verified by the presence of hydroxyapatite, even without osteoinductive factors. Capsules cultured under dynamic conditions presented an increased EC adhesion to microaggregates.

DISCUSSION & CONCLUSIONS:ASC-topodisc microaggregates proved to be optimal supports for EC adhesion. Mineralised topodisc-mediated microaggregates within liquefied-core capsules were obtained for static mono and co-cultures, even in the absence of osteoinductive factors. This was not observed for monoculture spherical particle conditions, highlighting topodiscs' nanogrooves as an impacting factor to induce osteogenesis. Next, we plan to study the microaggregates formed under dynamic conditions, assess the neo-vascularisation of the established co-culture, and the quality of the bone-like microtissue formed.

Acknowledgements:The authors acknowledge FCT for PhD grant SFRH/BD/129224/2017 and project CIRCUS (PTDC/BTM-MAT/31064/2017), CICECO-Aveiro Institute of Materials (UID/CTM/50011/2019) financed by national funds via FCT/MCTES, and European Research Council (ERC-2014-ADG-669858-ATLAS).

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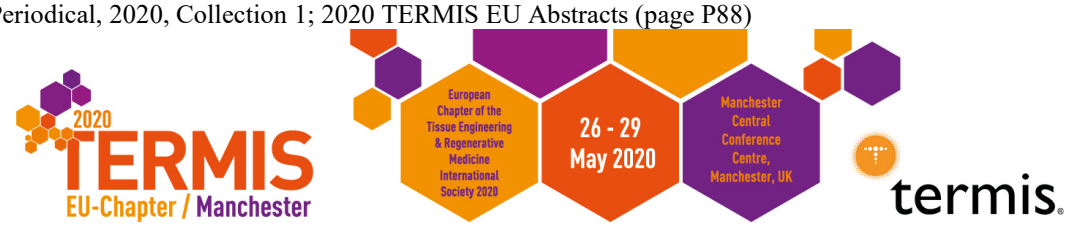
Keywords: Microenvironment and niche engineering, Bioreactors



Tissue Tension Prolongs Mitochondrial Functionality and Cellular Viability of Human Amniotic Membrane In Vitro

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INTRODUCTION:The human amniotic membrane (hAM) is a natural biomaterial, successfully used in tissue regeneration. Thus far, hAM has predominantly been applied in a decellularized form. More recently, promising potential of using vital hAM, containing viable amniotic cells has become centre of attention. While amniotic cells show beneficial stem cell properties, sustaining cellular viability of the hAM is challenging. Maintenance under common cell culture conditions results in rapid decrease of cell viability. It has been suggested that loss of tissue tension at term plays a role in the apoptosis of amniotic cells. Therefore, we investigated the impact of tensile strength on viability of amniotic cells in vitro, particularly on mitochondria-linked parameters.

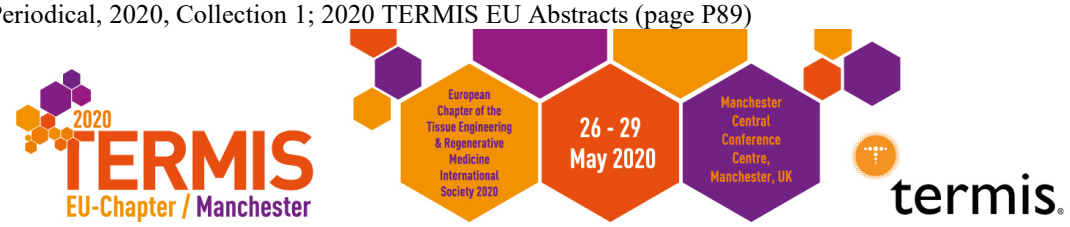
METHODS:Distended and non-distended hAMs biopsies were incubated for 7, 14 or 21 days. Cellular viability was measured with the EZ4U assay. Vital mitochondria-linked parameters, respiration and ATP synthesis were measured with high resolution respirometry and ATP Bioluminescence Assay Kit CLS II, respectively. Mitochondrial membrane potential was stained with tetramethylrhodamin-methylester (TMRM; 500 nM) and analyzed with inverted confocal microscope. Changes in mitochondrial morphology were analyzed with transmission electron microscope. Caspase 3 immunohistochemical staining was performed with anti-cleaved caspase 3 antibody. Gene expression of B-cell lymphoma 2-associated X protein (BAX) and B-cell lymphoma (BCL)-2 was determined by reverse-transcription quantitative PCR analysis.

RESULTS:Mitochondria-linked apoptotic gene expression of BAX/BCL-2 ratio was upregulated within 7 days in non-distended hAMs, followed by severe loss of mitochondrial internal structure and strongly increased caspase 3 expression at day 14. As a consequence, we observed loss of cellular viability at day 21. Contrary, in distended hAMs the mitochondrial function was preserved, caspase 3 expression increased only slightly and sequentially, the cellular viability was largely maintained.

DISCUSSION & CONCLUSIONS:The presence of tissue distention prolongs the cellular life span of hAM in vitro. We hypothesize that the cellular viability of hAM in vitro is regulated via mechano-sensing pathway. Upon loss of tissue tension, an unknown tension-driven mitochondrial pathway (TDMP) is activated, which ultimately leads to loss of viability of hAM cells in vitro. Further studies are required to delve into exact mechanisms connecting the tissue distention and viability of hAM cells. This knowledge will support optimization of hAM tissue cultures, since the distended hAM meets better the in vivo situation.

Acknowledgements:The study was funded by Austrian Research Promotion Agency (FFG; grant number 867674). J. Grillari is the co-founder of Evercyte GmbH.

Keywords: Biomaterials, Stem cells – general



Cell behaviour within nanogrooved sandwich-culture models

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INTRODUCTION:When cells are exposed to 2D or 3D environments, integrin-mediated adhesions are formed on one or both ventral and dorsal receptors.[1] Grooved surface topography and inherent cell contact guidance has been shown to influence cellular processes. However, studies rely on 2D approaches, which do not mimic the native tissue environment. To bridge the 2D-3D gap, sandwich-culture systems have been developed, where ventral and dorsal cell surface stimuli occurs simultaneously. To this end, nanogrooved or non-nanogrooved substrates were assembled with different combinations and relative orientations. We hypothesized that these varying sandwich-culture conditions would impact on cell behaviour.

METHODS:Polystyrene substrates were nanoimprinted using nanogrooved moulds. Plasma treated substrates were seeded with MC3T3-E1 cells. Afterwards, cell-seeded substrates were placed in contact with an unseeded substrate (single-seeding) or another previously seeded substrate (double-seeding) to close the SW. Different combinations were performed, namely groove orientation (0º or 90º), grooved/non-grooved assemblies, and gravity effect (upper or lower cell-seeded substrates).

RESULTS:Substrates presented homogeneous surface nanogrooves. Cell contact with both upper and lower substrates within the sandwich-culture was confirmed via focal adhesion staining. Cell elongation was effectively conditioned within sandwich-culture conditions when compared to control 2D substrates. Via F-actin staining combined with automated fibre alignment assessment, it was determined that for both single and double-seeded sandwich-cultures, cells acquired the orientation of the substrate on which they were seeded. Hence, for non-grooved surfaces, cell spreading occurred in a random fashion, whereas for grooved surfaces, a clear alignment was observed, independently of the stimuli offered by the opposing substrate.

DISCUSSION & CONCLUSIONS:The effect of nanogrooves on dorsal and ventral cell receptors under highly controlled orientation conditions was here pioneeringly explored. The significance of initial contact on cell morphology and orientation was highlighted, more so than further stimuli from opposing substrates. We have recently proposed nanogrooved microdiscs for a bottom-up cell-mediated 3D-aggregation where the formation of hydroxyapatite-containing microaggregates was observed even in the absence of osteoinductive factors.[2] Thus, studying the osteogenic differentiation within the proposed sandwich-cultures system could shed light on cell commitment, even with stimuli from differently oriented microdiscs. Hence, studying the differentiation of stromal cells into specific lineages under this controlled, quasi-3D sandwich-culture system could provide important knowledge on how biomechanical and biophysical cues impact cell commitment.

Acknowledgements:FCT for PhD-grant SFRH/BD/129224/2017 and project CIRCUS (PTDC/BTM-MAT/31064/2017), CICECO-Aveiro Institute of Materials (UID/CTM/50011/2019) financed by national funds through FCT/MCTES, and European Research Council (ERC-2014-ADG-669858-ATLAS).

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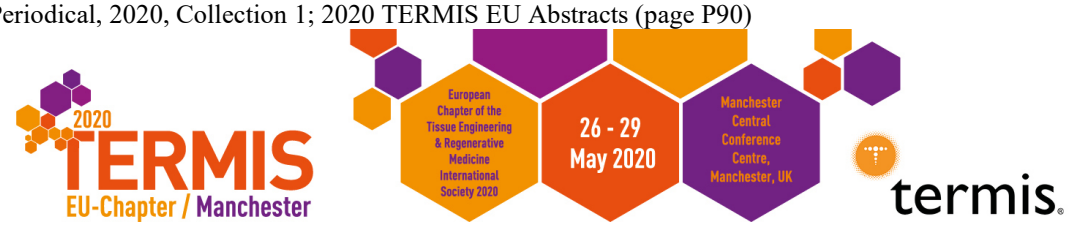
Keywords: In vitro microenvironments, Interfaces – engineered



Understanding the Cellular Mechanism Induced by Electrical Stimulation

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INTRODUCTION:The field of tissue engineering has faced several hurdles since its conception. A further barrier that is wholly pertinent to consider is the possibility of translating the stem cell therapies currently in development into commercially viable, scalable, and easily accessible treatments in the coming decades. Electrical stimulation, ES, holds promise for not only triggering early differentiation of human mesenchymal stem cells, hMSCs, but also guiding cells towards a certain pre-determined lineage

METHODS:Despite the increasingly encouraging results seen in the literature, the mechanism underpinning the differentiation of tissue due to ES is not yet understood. One area of interest is the interaction between ES, the production of reactive oxygen species, ROS, and tissue regeneration.3 Applying a DC ES regime of approximately 100mV/mm, delivered by 2.2V over a 22mm distance between platinum electrodes, for 60 minutes per day results in significant increases in metabolic activity of hMSCs ($p<0.001$), without a significant change in cell number, implying proliferation is not induced

RESULTS:A 30 minute per day regime does not induce the same effect. RNA extracted from hMSCs after 4 days of stimulation in osteogenic media under both temporal regimes was analysed using qRT-PCR. Both have significant upregulation of heme oxygenase, HMOX1, a downstream target of intracellular ROS, of up to 6 times versus the unstimulated control with respect to 18S, used as the reference gene.

DISCUSSION & CONCLUSIONS:Future work will involve studying the co-expression of RUNX2, the master regulator of bone formation, to explore if HMOX1 aids or inhibits osteogenic differentiation. It is hoped that this work will help to establish a greater understanding of the effects of ES, and unleash its potential to produce targeted tissue types in an affordable, reproducible manner.

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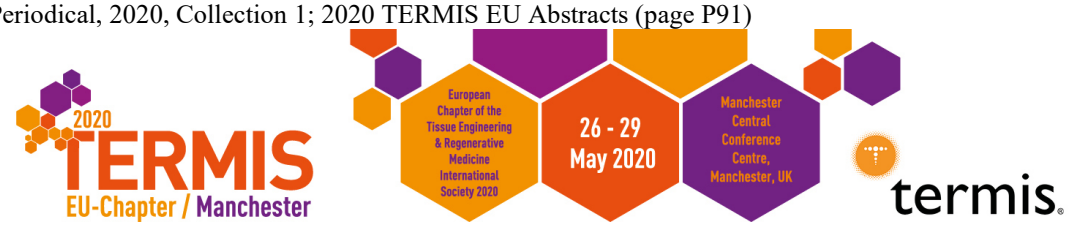
Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Multipotent (mesenchymal) stem cells



Long term stimulation of osteoblasts with low-frequency electrical fields

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INTRODUCTION:Bone deformation induce intrinsic voltages in bone and by using the inverse piezo effect, mechanical modification can be formed through an external electrical field. A clinical applied electroinductive screw system uses this effect to treat bone defects by applying electric and magnetic stimulation directly to the bone. Despite the positive results in clinical studies little is known about the fundamental processes acting during electrical stimulation of bone. Therefore, basic research needs to be done to understand the implication of electrical fields on cells involved in bone formation.

METHODS:The ASNIS III s screw served as a template to design a Ti6Al4V electrode for studying the effects of electrical fields in vitro in a 6 well system. Collagen coated coverslips were placed centred under each electrode and seeded with human osteoblasts. Cells were stimulated 3x45 min/d with a sinusoidal alternating voltage of 700 mV and 20 Hz over 28 days. During that time period, metabolic and alkaline phosphatase (ALP) activity, as well as calcification and the protein release were examined.

RESULTS:Using the mentioned settings (700 mV, 20 Hz) no changes in metabolic and ALP activity could be found. The calcification processes under electrical stimulation were donor dependent as alizarin red staining revealed different amounts of calcified matrix with electrical stimulation. A higher concentration of IL-6, DKK-1 and OPN in the supernatant could be found when cells were cultivated under electrical stimulation. Other bone remodelling marker proteins like OPG, RANKL, Leptin, BMP-2, IL-1β and TNF-α revealed no change in the concentration.

DISCUSSION & CONCLUSIONS:The investigations showed that long term electrical stimulation had no impact on the cell metabolism and on most of the typical bone remodeling markers. However, changes in IL-6, DKK-1 and OPN showed that the osteoblasts perceive the electrical field and that this field leads to changes in the cells. Therefore, further studies will be done with different voltages and frequencies to evaluate the best electrical field for bone formation. Besides, co-cultivation of osteoblasts and osteoclasts can give a more realistic in vitro model to study bone remodelling processes under the influence of electrical fields.

Acknowledgements:This study was supported by the German Research Foundation (DFG) JO 1483/1-1.

Keywords: Bone and bone disorders (osteoporosis etc),

Bioprinting Whole Intervertebral Discs to Understand Development and Inform Regenerative Therapies

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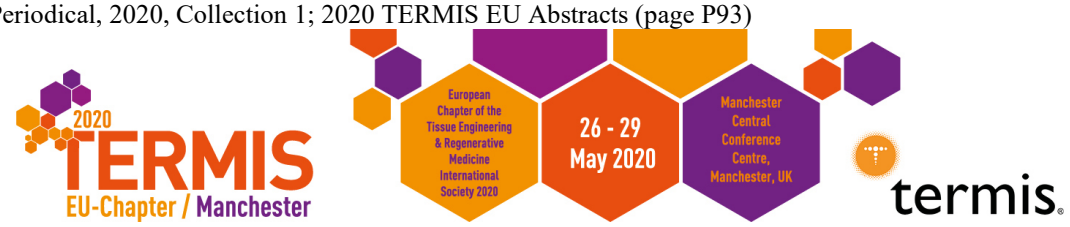
INTRODUCTION:Back pain costs the UK economy an estimated £12bn per year, and is driven largely by degeneration of the intervertebral discs (IVDs). The lack of effective clinical interventions regarding back pain, coupled with an ageing population, means that novel regenerative therapies are becoming essential. 3D Bioprinting can be used to accurately model and control the composition, structure and stiffness of the IVD and disc cell microenvironment, including the nucleus pulposus (NP) and annulus fibrosus (AF) regions. This has enabled research into the processes driving IVD development and degeneration, the results of which will help inform novel regenerative therapies.

METHODS:Fresh samples of human IVD tissue were mechanically tested to determine their compressive and tensile stiffness (ElectroForce 3310, TA Instruments). Immortalised lines of NP and AF cells were subsequently cultured on laminin-521 functionalised polyacrylamide gel-coated dishes of various physiologically relevant stiffnesses (1-12kPa) for seventy two hours. Cells were seeded at a density of 5000/cm2. qPCR analysis was conducted to assess the effect of stiffness on expression of phenotypic markers. Nuclear (DAPI) and F-actin (phalloidin) stains were additionally performed on separate cultures before imaging using 2D fluorescence microscopy. Image analysis software (CellProfiler, v3.1.8) was used to assess stiffness-induced morphological changes (cell area and eccentricity). Subsequently, NP and AF cells were cultured in 3D in printable alginate-collagen blended hydrogels for fourteen days and cell viability assessed using a live/dead assay on days three, seven and fourteen (Leica, SP8 Inverted Confocal Laser Scanning Microscope).

RESULTS:Statistically significant differences in disc cell morphology & phenotype were observed, with cell area increasing with substrate stiffness; interestingly this effect was more marked when using NP cells that were negative for CD24, a known notochordal cell marker. NP cells demonstrated good survivability in the 3D alginate-collagen gel environment.

DISCUSSION & CONCLUSIONS:This study has demonstrated that increasing substrate stiffness results in increasing NP and AF cell area and decreased eccentricity. The observation that the morphology of NP cells originating from similar lineages but expressing differential levels of CD24 are affected by substrate stiffness differently is an interesting one, which may be used to inform cell selection in cell-based regenerative therapies. The positive results of the live-dead assay for disc cells in 3D alginate-collagen gel cultures will allow further study of disc cell morphology and phenotype in 3D bioprinted gels of different stiffnesses and compositions.

Keywords: Intervertebral disc / spine and their disorders, 3D printing and bioprinting



Three-dimensional collagen matrix remodeling induced by human dermal papilla cells

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INTRODUCTION:The dermal papilla (DP) represents a unique mesenchymal cell cluster residing in the bulb of the hair follicle. DP induces the formation of a hair follicle in embryonic skin and supports its regeneration cycle in adults. DP cells embedded in a three-dimensional matrix may exhibit unique features and reorganize surrounding matrix in a different manner comparing to dermal fibroblasts. High density matrix resembles in situ surroundings of the hair follicle and is eligible for 3D bioprinting, however the properties of the high density matrix remains poorly investigated.

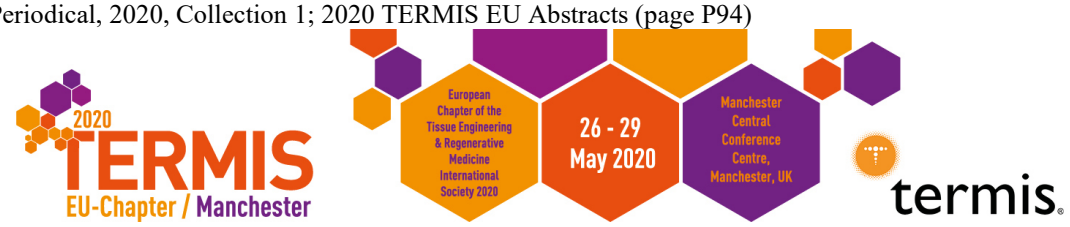
METHODS:In the current research, we studied the behavior of DP cells embedded into low or high density collagen matrix (from 5 and up to 30 mg/ml) compared to human dermal fibroblasts. For this purpose cells were seeded into collagen matrix (Viscoll, Imtek, Russia) at the concentration of 1 mln/ml and disk-shaped structures (gels) were fabricated by 3D bioprinting (3D Bioprinting Solutions, Russia). Following 2 and 4 weeks of in vitro cultivation mechanical characteristics of the cell-seeded collagen gels were investigated, as well as matrix contraction, cell survival and proliferation activity of the cells. The morphology and motility characteristics were recorded by time-lapse photography.

RESULTS:Obtained results indicate that cell-seeded collagen gels do not retain their original parameters of stiffness. Cells can strengthen stiffness of the high density collagen gels, and the contribution of DP cells to this process is lower than that of fibroblasts. Matrix contractility depends on the matrix density. The high density collagen gel of 30 mg/ml does not undergo contraction. We also show that that human DP cells retain high viability at all matrix densities investigated, but the proliferation activity decreases in high density matrices. Cells were found to extend dendritic protrusions to explore the neighborhood and communicate to each other regardless of the matrix density. Cell motility decreases with the increase in matrix density.

DISCUSSION & CONCLUSIONS:Further investigations are needed to evaluate the mechanisms by which DP cells and dermal fibroblasts reorganize the surrounding matrix.

ACKNOWLEDGEMENTS:The reported study was funded by RFBR according to the research project № 19-29-04060

Keywords: Skin, 3D printing and bioprinting



Development of an ex-vivo model for functional vascular tissue

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INTRODUCTION:Routine cardiovascular interventions, such as balloon angioplasty, cardiac bypass and stenting, provokes vascular activation and remodelling (1), often requiring further interventions. Research in this field is mainly based on complex, unrepresentative, unethical and expensive small animal models, or on primary vascular cells and static cultures of vascular rings. These methods improve experimental output but do not account for physical stimuli, such as flow and interaction with the extracellular matrix, essential for vascular tissue maintenance and function (2). This project aims to develop a versatile and economical bioreactor system for the long-term culture of large arteries in physiological conditions, and the study of pathological remodelling.

METHODS:A novel perfusion system was designed in house and 3D printed by Multijet-Fusion. Porcine carotid arteries were excised and cultured using our perfusion system at different flow rates (10-140 ml/min), or under static culture, for up to 7 days. Balloon injury was induced in some samples at day 0. Tissues were functionally tested in response to vasoconstriction and vasodilation stimuli, and analysed by immunofluorescence and confocal microscopy.

RESULTS:We produced a cost-efficient and versatile 3D printed vascular bioreactor system to culture blood vessels of different origin and size. The system fits on standard centrifuge tubes and can be efficiently multiplexed, allowing parallel cultures, whilst minimal media consumption renders it amenable to pharmacological studies. Perfusion cultured porcine carotid arteries at days 3 and 7 showed reduced apoptosis in the media and intima layers of the vessel wall, compared to static controls. Endothelial coverage and functionality was preserved for up to 7 days, as shown by histological and pharmacological analysis. Culture in high flow conditions reduced pathological activation of the smooth muscle cells, whilst maintaining a significantly higher cell alignment, when compared to the low-flow and static cultures.
Upon balloon injury, tissues cultured in flow recapitulated the hallmarks of remodelling, such as intimal denudation, smooth muscle cell disarray and activation.

DISCUSSION & CONCLUSIONS:These results suggest that our robust and controlled culture system could be exploited to study the mechanisms of vascular complications ex-vivo. This work lays the basis for future investigations into how vessel wall resident cells contribute to the pathological remodelling of blood vessels.

ACKNOWLEDGEMENTS:This project has been funded by the University of Surrey. We would like to thank the Pirbright Institute for providing the porcine tissues used in this research.

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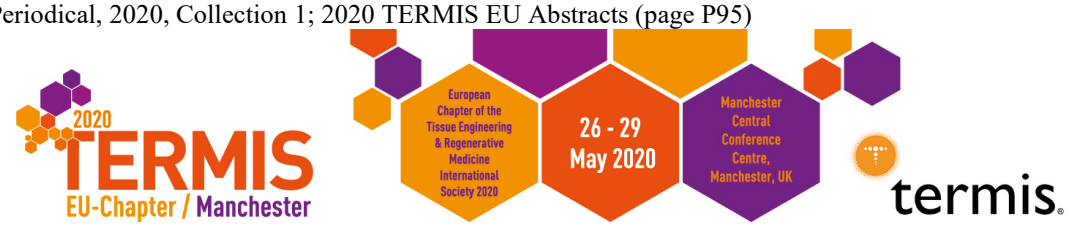
Keywords: Cardiovascular, Vascular systems / vascularisation and heart



Osteochondral tissue co-cullture: An in vitro and in silico approach

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INTRODUCTION:Osteochondral defects from osteoarthritis or trauma place a huge burden on the healthcare sector globally. Osteochondral tissue engineering aims to regenerate functional and physiologically relevant cartilage and its subchondral bone tissues. Given the distinct structural and biochemical difference between bone and cartilage, layered scaffolds and bioreactors are commonly employed. This study investigated the osteochondral tissue growth on a novel 3D printed bi-layered polylactic acid (PLA) scaffold in a dual-chamber perfusion bioreactor in vitro, and the microenvironment inside the co-culture system through in silico simulation.

METHODS:The top chondral layer of the scaffold was composed of a coarse mesh of 1000 µm diameter PLA struts infiltrated with type I bovine collagen whilst the bottom osseous layer was composed of a fine mesh of 500 µm diameter PLA struts. Mouse chondrogenic ATDC5 and osteoblastic MC3T3-E1 cells were seeded onto the respective chondral and osseous layer of the scaffold. They were dynamically cultured in a dual-chamber perfusion bioreactor at 0.02 ml/min flow rate for 7 days in the respective chondrogenic and osteogenic media. Live/Dead, resazurin assay, scanning electron microscope (SEM) and fluorescence cell tracking experiment were conducted. Also, finite element models (FEM) based on the microcomputed tomography image (µCT) of the manufactured scaffold as well as on the computer-aided design (CAD) scaffold were constructed; the microenvironment inside the two FEM was investigated.

RESULTS:After perfusion, Live/Dead assay and SEM results showed viable cells attached on both chondral and osseous layers; Resazurin assay revealed a ~3-fold increase in cell metabolic activity; from the fluorescence cell tracking study, increased number of ATDC5 and MC3T3-E1 cells were observed on the respective chondral and osseous layers. In silico results showed that the CAD and the actual manufactured scaffold had significant differences in the flow velocity, differentiation media mixing in the bioreactor and fluid-induced shear stress experienced by the cells.

DISCUSSION & CONCLUSIONS:In conclusion, the in vitro study showed that the co-culture system supported osteochondral tissue growth in terms of cell viability, proliferation, distribution and attachment, whilst the in silico simulation revealed the desirable microenvironment provided by the co-culture system including adequate mass transport, limited differentiation media mixing and mechanical stimulation. This co-culture system can potentially be used as an inexpensive tool for testing newly developed pharmaceutical products for osteochondral defects.

Acknowledgements:This work is financially supported by Rosetrees Trust M484 grant and BBSRC BB/M013545/grant.

Keywords: In vitro microenvironments, In silico models

A 3D fluid-dynamic cancer model to resemble the in vivo drug administration as a new platform for drug testing

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INTRODUCTION:The integration of 3D human tumor organoids with fluidic cell culture chambers allows to mimic the in vivo organ-organ fluidic connections and drug systemic administration, finally offering a reliable and fast approach for novel molecules prescreening tests. In this work, 3D cell-laden hydrogels have been properly realized as ovarian tumor models and cultured within a fluidic Multi In vitro Organ-MIVO®-device for in vitro resembling the cancer environment and carry out drug efficacy assays, alternative to animal preclinical models, by monitoring the cancer regression over time.

METHODS:Human ovarian cancer cell line (SKOV-3) has been used to realize alginate-based 3D cell-laden hydrogel-based tumor models, as reported (1). 3D tumor tissues have been cultured within MIVO® chamber under fluid flow resembling the capillary blood flow feeding the tumor. Drug (cisplatin) efficacy have been evaluated in MIVO resembling the in vivo systemic drug transport mechanisms and compared with (i) the in vitro tissue culture under static condition, and with (ii) the use of xenograft mouse model. In particular, SKOV-3 viability was quantitatively assessed through Alamar Blue Assay at different time points. Immunostaining for apoptosis and cell proliferation was also carried out. For in vivo tests, SKOV-3 derived tumors were established via subcutaneous injection into the right flank of nude mice. Tumor size was monitored over time. After 10 days, mice were randomized into two treatment groups when tumor volumes increased to 50 mm3. Cisplatin was administered intravenously every seven days for 3 weeks. Tumor growth was quantified three times a week by using a digital caliper.

RESULTS:After one week of drug treatment, the viability of SKOV-3 cultured within the 3D hydrogels in MIVO® decreased up to 50%; caspase positive cells were observed homogeneously distributed within the 3D gels, while very few proliferating cells were observed after 1 week of drug treatment. On the contrary, under static conditions the cells were still viable (higher than 80%) and proliferating until 7 days of drug treatment highlighting chemoresistance. Exceptionally under MIVO® tissue culture, the in vitro tumor regression kinetics resembles exactly the tumor mass reduction measured in vivo.

DISCUSSION & CONCLUSIONS:These results highlight that the in vivo-like dynamic environment provided by the MIVO® device allows to properly resemble the 3D tumor tissue perfusion, its culture under a capillary circulation and the systemic drug transport mechanism, suggesting its potential as reliable and cruelty-free platform for preclinical drug efficacy tests.

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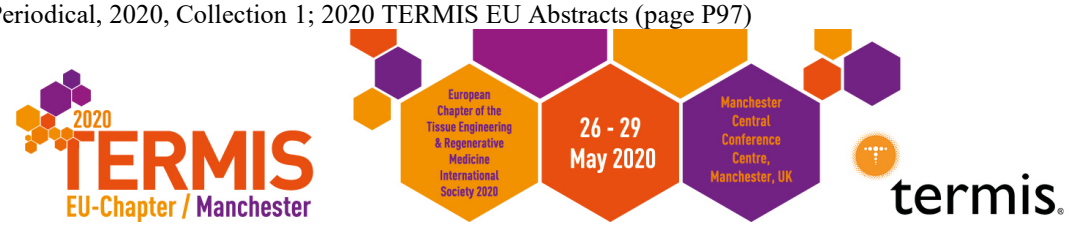
Keywords: Disease models,



Mechanical stimulation for tissue engineering: characterising load-induced changes by the 'collagen barcode'

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INTRODUCTION:Differentiated cells can be characterised by the composition of collagen isoforms that they produce in response to specific mechanical loads – effectively a 'collagen barcode' that functionally defines engineered tissues. Collagen is one of the major structural proteins within the body, currently known to exist as 28 different isoforms that are each associated with specific functions in the tissue, such as mechanical resilience, structure, proteoglycan-binding and cell fate regulation. The combinations and ratios of collagen types vary across all tissues, e.g. Type I, III, V, XI, XII, XIV in tendon; Type II, IV, V, VI, IX, XI in cartilage.

METHODS:We have used 3D printing to generate bespoke bioreactor components which apply mechanical stimulus to cells seeded within 3D hydrogels. We have customised our culture components around an EBERS TC3 bioreactor and replicated individual chambers at low cost. The mechanical stimuli applied is cyclic tensile force at 3, 5 and 10% strain, 1Hz for 5 hours per day over 3 weeks. We analysed media and hydrogel samples using western blot, qPCR, TEM and two-photon to visualise the structure.

RESULTS:Our early results show that in response to cyclic tensile loading, hMSCs alter the collagen composition of the extracellular matrix they produce. Tensile testing shows an increasing trend with higher strain rates (3, 5 and 10%), however collagen genes do not see a significant change in regulation, indicating that the strain rates have a greater effect on the structural properties rather than the molecular properties.

DISCUSSION & CONCLUSIONS:Defining 'optimal' loading conditions may help generate engineered tissues with comparable ratios of collagen types as found in healthy native tissue, thus improved functionality and integration as implants. Using total collagen isoform expression to characterise these tissues in comparison to native, functional tissue is a novel approach which sheds light on developmental processes recapitulated in tissue engineering allowing us to better define successful bioengineered implants.

Acknowledgements:Financial support was received from the Institute of Ageing and Chronic Disease, University of Liverpool.

Keywords: Bioreactors, Musculoskeletal (inc ligament / tendon / muscle / etc)

Liquefied and multilayered capsules as bioreactors to regulate stem cell fate

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INTRODUCTION:Biochemical and biophysical properties of materials provide various mechanical cues influencing cellular behavior. In particular, the viscosity of the extracellular matrix (ECM) can affect the cellular differentiation pathway. The physical interaction of cells with their microenvironment is more precisely mimicked in 3D systems. Herein, we propose our well-established cell encapsulation system of liquefied and multilayered capsules (LMC) [1], to expose cells to variable core viscosities. Improving the understanding on how viscosity influences cell response will provide a further tool to manipulate the fate of stem cells.

METHODS:Three different concentrations of alginate, namely 0.5%, 0.75%, and 1% w/v, were prepared. The viscosity was evaluated by rheometer analysis. Alginate macro-beads encapsulating adipose stem cells (ASCs) and polycaprolactone surface-modified microparticles were produced by extruding low, medium, or high viscosity alginate solutions through a 21G needle in calcium chloride bath. Alginate macro-beads were then used as templates to produce a multilayered membrane by Layer-by-Layer (LbL) technique. After a mild core liquefaction process with EDTA, LMC were cultured in basal or osteogenic medium under a dynamic environment, to better mimic the dynamic environment of native tissues.

RESULTS:According to the rheometer analysis, the three concentrations of alginate solution presented significantly different viscosities. Light microscopy results showed the formation aggregates composed by cells and microparticles within the core of LMC after 21 days of culture in both media. Larger aggregates were found in LMC cultured in osteogenic medium. The fluorescence staining of F-actin filaments demonstrated the interaction and structural organization of the encapsulated cells with the microparticles inside the viscous environment of LMC with 1% core-alginate. MTS assay showed that ASCs remained viable up to 21 days of culture.

DISCUSSION & CONCLUSIONS:We demonstrated that highly viscous core environment allowed the encapsulation of living cells under a dynamic environment up to 21 days. Therefore, we anticipate that the remaining less viscous alginate cores with 0.5% and 0.75% will not jeopardize the viability of the encapsulated cells. Our next step is to use the developed macro-bioreactor system to evaluate its influence on the differentiation pathway of ASCs.

ACKNOWLEDGEMENTS:The authors acknowledge funding from FCT for the doctoral grant (SFRH/BD/147418/2019) and project CIRCUS (PTDC/BTM-MAT/31064/2017), and from the European Research Council for project ATLAS (ERC-2014-ADG-669858-ATLAS). This work was developed within the scope of project CICECO-Aveiro Institute of Materials (UIDB/50011/2020,UIDP/50011/2020), financed by national funds through FCT/MEC and when appropriate co-financed by FEDER under PT2020 Partnership Agreement.

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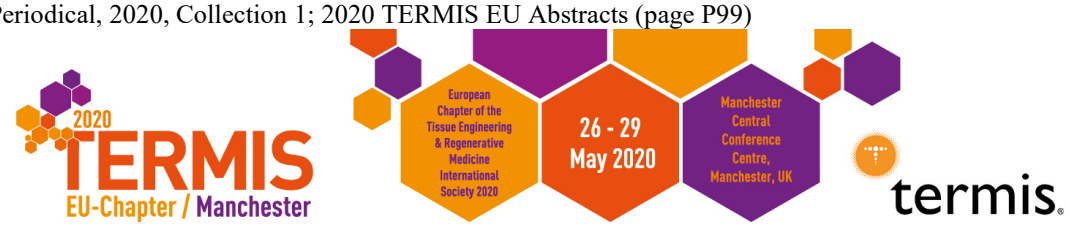
Keywords: Differentiation, Microenvironment and niche engineering



Dynamic culture of Adipose Derived Stromal Cells in bioreactor for construction of an artificial urinary conduit – an in vitro study

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INTRODUCTION:Bladder cancer is the 10th most common cancer worldwide. About 20% of bladder cancers are muscle-invasive and are indication for a radical cystectomy. The use of ileal segment is a standard method for urinary diversion after bladder removal. Use of this method extend surgical procedure and lead to numerous metabolic complications. To overcome these side effects, tissue engineering methods can be utilized to create an artificial urinary conduit. The aim of this study was to develop a method of the tissue-engineered conduit construction for urinary diversion using dynamic bioreactor culture.

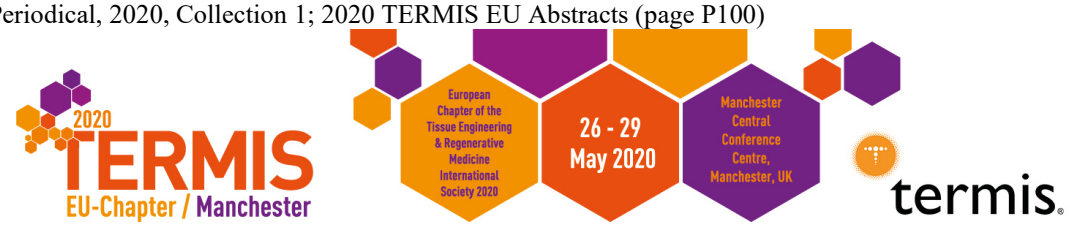
METHODS:Tissue engineered tubular scaffolds were used for construction of the artificial urinary conduits (n=40). Conduits were seeded manually with adipose-derived mesenchymal stromal cells (~$10x10^6/cm^2$) isolated from porcine fat tissue. Cells were grown on scaffold surface in a bioreactor for 6 days. During the culture pH, pO2 and temperature were continuously monitored. On the basis of culture parameters and glucose values measured each day medium feeding rate was established. ADSCs were seeded after 3rd or 4th passage, before seeding on scaffold mesenchymal stromal cell phenotype was analyzed using flow cytometry (positive markers: CD29, CD44, CD90; negative markers: CD11b, CD31, CD45). After end of experiment analysis of cell growth on scaffold surface was performed using scanning electron and confocal microscopy. Cells were detached from scaffold surface in order to check their number and changes in phenotype after dynamic culture in bioreactor.

RESULTS:ADSCs expressed typical for mesenchymal stem cells markers. Scanning electron microscopy revealed appropriate morphology of cells grew on artificial urinary conduit. Cells were elongated, spindle-shaped with good condition confirmed by production of microvesicles. Analysis using confocal microscopy showed that majority of cells seeded on artificial urinary conduit after the end of 6-days culture were viable. Analysis of culture medium and bioreactor vessel showed that only small number of cells (<10%) detached from tested material during dynamic culture in bioreactor. Cells detached from scaffold surface showed significant changes in cell phenotype in the case of CD29 antigen.

DISCUSSION & CONCLUSIONS:Dynamic culture of tissue-engineered scaffold seeded with ADSCs in bioreactor enables their proper growth. Application of such culture method may allow for construction of artificial organs for clinical application.

Acknowledgements:The present work was supported by the National Centre for Research and Development (NCBR) in Poland under Agreement no. STRATEGMED1/235368/8/NCBR/2014 (Smart AUCI Project) within the Strategic Programme STRATEGMED “Prevention practices and treatment of civilization diseases.”

Keywords: Multipotent (mesenchymal) stem cells,



3D Tissue engineering and vascularized bioreactor development

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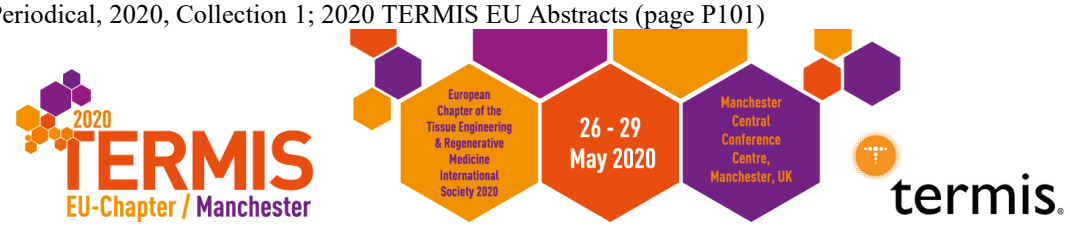
INTRODUCTION:3D cell culture systems with bioreactors containing hydrogels have been widely used for tissue engineering. The biggest possible size of these hydrogel constructs is limited to the diffusion of nutrients and O2. In this project, we present a hydrogel model with embedded and modifiable vascular networks, enabling the production of large hydrogels with increased cell viability. Since the environment of cells in this 3D bioreactor system is much closer to the in vivo situation compared to standard 2D cell culture in plastic flasks, this is an alternative and improved model for advanced in vitro research.

METHODS:Bioreactor design For the design of the bioreactor, we use the open source software blender or FreeCAD. For 3D printing, we use the 3D printer Ultimaker 3 with extra fine settings (layer height 0.06 mm, 100% infill and 60 mm/s print speed). All bioreactors were produced with a non-toxic and biocompatible polymer PLA (polylactic acid). 3D cell culture For 3D cell culture, we use the self-designed bioreactor including hydrogel and cells. The reactor is connected to a perfusion system on both sides under sterile conditions and the system is filled with nutrient medium (DMEM + 10% FCS + 1% P/S). The following flow parameters were set inside the software: 15mbar, 10s unidirectional flow. Cells were cultured at 37°C, 5% CO2 and 95% RH.

RESULTS:Mesenchymal stem cells were mixed successfully with scaffold material and inserted into a bioreactor. A pump system guaranteed medium flow and nutrient supply. Vital cells were observed by fluorescence microscopy and fabrication of vascular structures was successfully obtained by molding forms. Cross sections of hydrogels indicated a time dependent nutrient penetration of the hydrogel. Cell vitality decreased gradually with increased distance from the channel. Furthermore, a 2-cell-layer approach showed a notable cultivation of green (inner layer) and red fluorescent cells (outer layer) in one bioreactor, indicating a successful step for culturing more complex organ structures.

DISCUSSION & CONCLUSIONS:This new and innovative technique of 3D tissue engineering provides a cost-effective, reproducible, controlled and fast approach to produce 3D tissues with vascular structures inside the hydrogel. This research project will be the basis of further projects, achieving bigger tissues with more complex vessel-like structures. It will also enable research topics to labs without sophisticated equipment and no access to animal models to design and produce vascularized tissues in their own bioreactors.

Keywords: Bioreactors, Vascular systems / vascularisation and heart



High hydrostatic pressure as a new approach for processing allogenic bone grafts

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INTRODUCTION:Due to an increasing number of head and neck tumors in recent years, the need for bone substitutes is increasing. Although autologous tissue material is able to integrate well into the bone the availability is limited. Synthetic materials are easily available, but their mechanical properties are often insufficient and they can trigger recipient's immunological reactions. The treatment of allogenic bone tissue with high hydrostatic pressure (HHP) is a new approach to process allogenic grafts resulting in a good biocompatibility and maintaining mechanical properties. For this study, we either treated human osteoblasts (hOBs) or cylindrical bone specimens with differing HHPs to determine devitalization efficiency or induced cell death. In addition mechanical properties and residual DNA content of bone cylinders were analyzed.

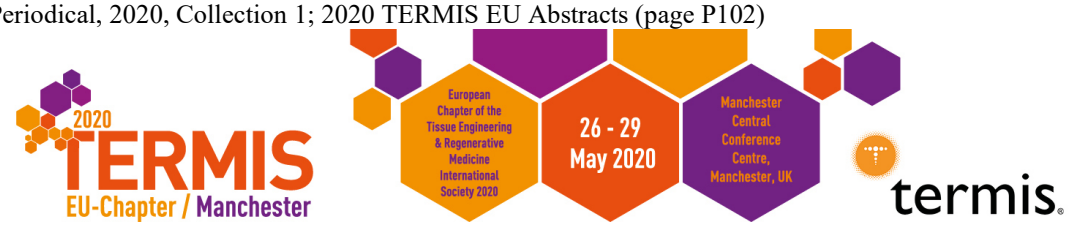
METHODS:For cell experiments, hOBs were isolated from femoral heads and cultivated under standard culture conditions. Cells were harvested to produce spheroids which were treated with different HHPs ranging between 100-150 MPa, 250-300 MPa and 450-500 MPa. The spheroids were cultured for at least 24 h. Necrosis and apoptosis detection was determined by ELISA and flow cytometry, respectively. Moreover, metabolic activity was analyzed by means of WST-1 assay. Bone cylinders, harvested from the distal epiphysis of the femur, were treated with HHP (250-300 MPa).They were used for compression testing to determine the stiffness and strength and DNA content was analyzed.

RESULTS:After HHP treatment metabolic activity of osteoblasts decreased, whereas HHP of 250 MPa showed higher effects compared to HHP of 150 MPa. The highest HHP range (450-500 MPa) induced only necrosis, whereas HHP from 250-300 MPa induced both apoptosis and necrosis equally. Based on the preliminary results of the cell experiments, bone cylinders where treated with 250-300 MPa to devitalize cells. Here, a clear decrease in the DNA amount was observed, while mechanical properties between controls and HHP-treated bone cylinders were similar.

DISCUSSION & CONCLUSIONS:The level of applied HHP seems to be connected to the necrosis-apoptosis ratio and influences the metabolic activity. The data show, that HHP devitalizes tissue while matrix integrity and mechanical properties of the tissues were not affected. HHP treatment therefore appears to be a good alternative to existing methods for providing tissue replacement materials. Although further studies have to be carried out regarding tissue revitalization and immunological effects.

Acknowledgements:This joint research project HOGEMA is supported by the European Social Fund (ESF), reference: ESF/14-BM-A55-0012/18 and the Ministry of Education, Science and Culture of Mecklenburg-Vorpommern, Germany.

Keywords: Decellularised matrices, Biomaterials



Tissue engineered scaffolds for mimetic autografts

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INTRODUCTION:Bone tissue has an important intrinsic regenerative capacity. However, this regeneration can be compromised, leading to delayed fracture healing and nonunion. Due to the scarcity of bone tissue that can be used as autograft, novel tissue engineering strategies arise as a promising solution by using biocompatible materials which can be functionaliz³ed with cells and morphogens

METHODS:We designed polycaprolactone (PCL) based autografts composed of an inner cylindric scaffold, produced by PCL extrusion, providing mechanical stability and an osteoconductive environment. We also created a periosteum mimetic membrane (MA) by the addition of an outer thin and highly porous fibrillar PCL tube, synthesized by melt electrowriting (MEW). To assess their regenerative capacity and biocompatibility, these scaffolds were placed in critical size femur defects in rats and compared with untreated rats (Empty). Ten weeks after surgery µCT and histological studies were carried out. Additionally, we are focusing on the generation of different PCL biocomposites, such as poly ethyl-acrylate (PEA) covered PCL membranes which can enhance morphogen functionalization, reducing the effective BMP dose; or as poly-lactic-glycolic acid combined with poly-ethylene-glycol (PLGA-PEG) nanoparticles coupled to our PCL membrane to develop advanced drug delivery systems offering controlled liberation of morphogens over time.

RESULTS:At the µCT level, structural mimetic PCL scaffolds, devoid of cells and morphogens, showed no significant differences in healing or bone formation (Empty group, 11.47±4.93 mm3; MA, 14.95±3.09 mm3, p=0.1711). Histological analysis demonstrates that MEW PCL mimicking periosteum enhances bone growth and present good implant integration, but insufficient for successful healing. However, when functionalized with rhBMP-2, PEA-PCL implants show efficient bone regeneration, X-ray analysis reveals that 80% of animals (n=5) treated presented radiographic healing or substantial bone synthesis in the bopne defect. In vitro, we determined that the morphogen dose was very low, 55.64±14.83 ng (n=6).

DISCUSSION & CONCLUSIONS:In conclusion, acellular mimetic autografts need to be optimized by functionalization with morphogens (BMP-2, BMP-7) and/or mesenchymal progenitor cells (PMSC). Generation of biocomposite materials of PCL-PEA shows promising results in bone regeneration by using 100 folds lower dose than typically described.

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Keywords: Biomaterials, Biologics and growth factors

Modulating Glucose Concentration in a Fully Defined Medium for Successful in vitro Osteogenic Differentiation

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INTRODUCTION:Fetal bovine serum (FBS) is a common supplement in cell culture providing cells with growth factors, hormones and vitamins1 but is controversial due to its unknown batch-dependent composition. Alternatively, chemically defined animal-component-free (ACF) media are available for different cell types. Their exact formulations are not provided. In bone tissue engineering, mesenchymal stromal cells (MSCs) should differentiate towards the osteogenic lineage, produce collagen and deposit a mineralized matrix. Glucose is one essential component in cell culture medium that has an effect on osteogenic differentiation and also depends on the type of FBS used.2 The goals of this study were i) to develop a fully defined medium devoid of FBS and ii) to compare the effect of different glucose concentrations in this fully defined medium on the osteogenic differentiation of MSCs n vitro.3

METHODS:The defined medium was prepared by adding essential components for cell survival, growth and differentiation to DMEM/F12 basal medium. Human bone marrow-derived MSCs were grown as pellets in either defined medium or 10 % FBS-containing medium for three weeks with low (5mM) or high (25mM) glucose concentrations. Osteogenic differentiation was induced by adding dexamethasone, ascorbic acid and β-glycerophosphate to the media. Picrosirius Red and Alizarin Red staining were used to study collagen deposition and mineralization and as well as specific markers for bone cells.

RESULTS:In previous experiments with ACF, osteogenic cell differentiation was limited and mineral formation did not occur, suggesting that ACF is suboptimal for bone tissue engineering. In our defined medium, cells survived and differentiated towards the osteogenic lineage and deposited a collagenous and mineralized matrix over three weeks; however, mineral formation was more prominent in the low glucose concentration medium. These results were in agreement with the FBS-containing medium.

DISCUSSION & CONCLUSIONS:Developing a medium with defined composition needs to be optimized for specific cells types and applications. This will enable basic research on the physiology of the cells and the cellular behaviour by varying concentrations of soluble parameters, which is currently hampered by the use of FBS.

Acknowledgements:Financial support by the Dutch Ministry of Education, Culture and Science (Gravitation Program 024.003.013) is gratefully acknowledged.

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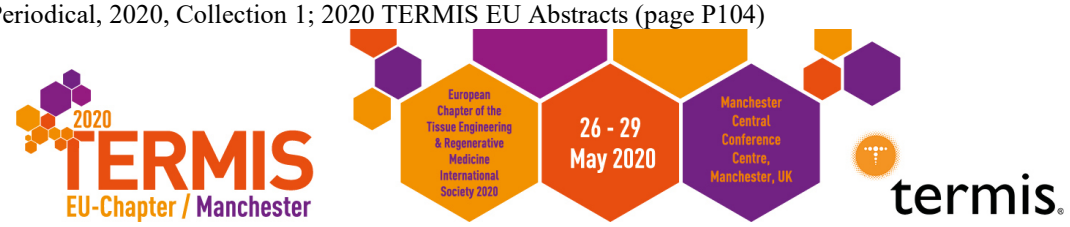
Keywords: Bone and bone disorders (osteoporosis etc), Differentiation



Osteogenic activity of Actifuse™ is not enhanced by pre-adsorbed BMP2

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INTRODUCTION:Silicate-substituted hydroxyapatite (SA) has a greater affinity for BMP2 than stoichiometric hydroxyapatite (HA) [1], leading to the hypothesis that the enhanced osteoconductivity of SA bone graft substitutes (BGS) is linked to surface enrichment of BMP2 from the host. To test this, we assessed the proliferation and osteogenic differentiation of human mesenchymal stem cells (hMSCs) on SA-BGS in the presence and absence of BMP2.

METHODS:hMSC were cultured on 150mg of 80% porous 0.8wt% SA-BGS 1.0-2.0mm granules (Actifuse™, Baxter Inc, AF) with (AF+BMP2) or without (AF) pre-adsorbed BMP2 for up to 21 days. For controls, hMSCs were cultured on tissue-culture treated plastic in the absence of both AF and BMP2 (control) or in the presence of 300ng BMP2 (control+BMP2). AF+BMP2 was generated by incubating 150mg AF in 1mL media supplemented with 300ng BMP2 for 2h. Adsorption was calculated by measuring free BMP2 using ELISA. Proliferation of hMSCs was assessed by quantifying total DNA. Osteogenic differentiation was assessed by quantitative RT-qPCR of osteogenic genes, analysis of ALP activity, and procollagen type 1 production.

RESULTS:72±9ng BMP2 was adsorbed onto AF+BMP2. From Days 3-21, DNA levels were significantly higher on AF than on AF+BMP2, suggesting with AF present, BMP2 suppressed proliferation. Control+BMP2 promoted similar specific ALP activities compared with control, whereas AF+BMP2 resulted in higher specific ALP activity over 21 days. hMSCs on AF demonstrated an increasing trend, and significantly highest specific ALP activity compared to all samples. Both AF and AF+BMP2 significantly stimulated procollagen production from Days 7-21 compared to control and control+BMP2, suggesting the upregulation was based on the presence of SA-BGS. Samples containing BMP2, control+BMP2 and AF+BMP2, showed significant upregulation of osterix expression compared with control and AF; control+BMP2 having the highest expression. This suggests a stronger osteogenic effect of BMP2 in solution than when bound. The opposite was found for endogenous BMP2 expression: AF+BMP2 promoted the significantly highest expression from Days 1-21.

DISCUSSION & CONCLUSIONS:Incubation on AF supported osteogenic differentiation of hMSCs compared with control. AF+BMP2 and control+BMP2 significantly stimulated early osteogenic gene expression, but it did not translate into increased osteogenic behaviour in the study time frame. Therefore, the enhanced SA-BGS osteoconductivity might not be linked to surface enrichment of BMP2 from the native host in isolation.

Acknowledgements:The authors thank Baxter Inc. and Queen Mary University of London for funding the studentship of J.K-S and Baxter Inc. for materials supply.

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Keywords: Multipotent (mesenchymal) stem cells, Differentiation

Bioactive membranes for the future treatment of osteoporotic fractures

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INTRODUCTION:Fragility fractures are the main consequence of osteoporosis and their treatment remains a challenge in the orthopedic field. Evidences demonstrate that an impaired periosteal activity is responsible for recurrent fractures. We thus suggest the development of a natural-based regenerative membrane fixing biological active capsules for periosteum regeneration purposes. Here, a laminarin hydrogel was microfabricated with suitable mechanical properties and adequate resorbable times for bone regeneration. This membrane acted as on-site fixing agent for biological active capsules and is expected to be implanted by wrapping the membrane around the defect to guide bone regeneration.

METHODS:Liquified capsules were produced following well-stablished procedures in the group,[1,2] and comprised i) microparticles for cell adhesion and for the transport of osteogenic-differentiating factors, and ii) a cell niche of ASCs and HUVECs. Cells and microparticles were resuspended in a sodium alginate solution. Alginate microgels (capsules) were produced by electrohydrodynamic spraying. Then, layer-by-layer was performed using 3 different polyelectrolytes. The process was repeated until a 10-layered membrane was created. Methacrylated laminarin (MeLam) hydrogels were produced by bringing a solution of MeLam in contact with an optimized PDMS master,[3] followed by exposure to UV irradiation. Capsules with optimized sizes were entrapped within the micropillars of the laminarin membrane. To avoid the rupture of the capsules, the liquefied core was only obtained after capsules' entrapment within the laminarin-based membrane via chelation with EDTA. Membranes and capsules were physically and chemically characterized. In vitro osteo- and angiogenic potential was also assessed.

RESULTS:Fluorescent microscopy revealed that the micropatterned membranes were able to recapitulate the geometry of the master mold, maintaining the micropillars integrity throughout the fabrication process. The entrapment efficiency of the bioactive capsules in the micropatterned hydrogel was investigated achieving higher entrapments when capsules diameters matched the micropillars spacing. The bioactive membrane (micropatterned hydrogel+capsules) was placed onto a 6-well plate filled with culture medium (capsules facing down), in an orbital shaker and incubated over a period of 21 days. The results demonstrated that the capsules were released from the membranes in the first days of culture. Also, cells remained viable and osteogenic differentiation was accomplished.

DISCUSSION & CONCLUSIONS:A bioinspired and natural-based membrane was microfabricated to accommodate bioactive capsules for periosteum regeneration purposes. The membrane is expected to induce regional bone formation and an overall stimulation of bone regeneration.

Acknowledgements:We thank the financial support of ERC-2014-ADG-669858 for the project "ATLAS" and the FCT project "PROMENADE" (PTDC/BTM-MAT/29830/2017).

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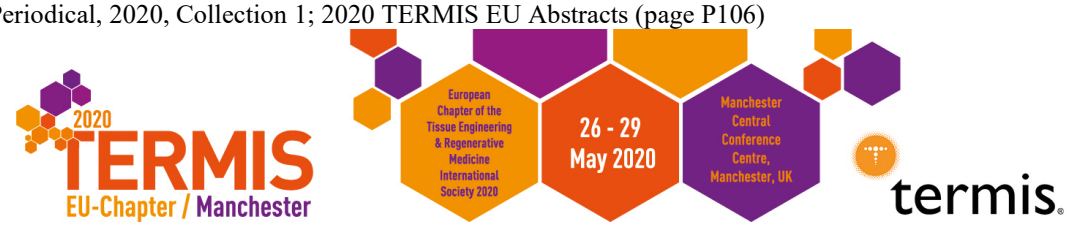
Keywords: Bone and bone disorders (osteoporosis etc), Hydrogels and injectable systems



Engineered extracellular matrix enhances the bone regeneration potential of aged human bone marrow stromal cells

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INTRODUCTION:Regeneration of bone defects in elderly patients is limited due to the decreased function of bone forming cells and compromised tissue physiology. Previous studies suggested that the regenerative activity of stem cells from aged tissues can be enhanced by exposure to young systemic [1] and tissue microenvironments [2]. The aim of our project was to investigate whether extracellular matrix (ECM) engineered from human induced pluripotent stem cells (hiPSCs) [3] can enhance the bone regeneration potential of aged human bone marrow stromal cells (hBMSCs).

METHODS:ECM was engineered from hiPSC-derived mesenchymal-like progenitors (hiPSC-MPs), as well as young (<30 years) and aged (>70 years) hBMSCs. ECM structure and composition were characterized before and after decellularization using immunofluorescence and biochemical assays. Three hBMSCs of different ages were cultured on engineered ECMs. Growth and differentiation responses were compared to tissue culture plastic, as well as to collagen and fibronectin coated plates.

RESULTS:Decellularized ECMs contained collagens type I and IV, fibronectin, laminin and < 5% residual DNA, suggesting efficient cell elimination. Cultivation of young and aged hBMSCs on the hiPSC-ECM in osteogenic medium significantly increased hBMSC growth (days 5 to 42) and markers of osteogenesis, including collagen deposition (day 21), alkaline phosphatase activity (day 21), bone sialoprotein expression (day 21) and matrix mineralization (day 42) compared to plastic controls. In aged BMSCs, matrix mineralization was only detected in ECM cultures in osteogenic medium. Comparison of ECMs engineered from hiPSC-MPs and hBMSCs of different ages suggested similar structure, composition and potential to enhance osteogenic responses in aged BMSCs. Contribution of specific matrix components and underlying mechanisms need to be further elucidated.

DISCUSSION & CONCLUSIONS:Our studies suggest that aged BMSCs regenerative activity can be enhanced by culture on engineered ECM. HiPSCs represent a scalable cell source, and tissue engineering strategies employing engineered ECM materials could potentially enhance bone regeneration in elderly patients.

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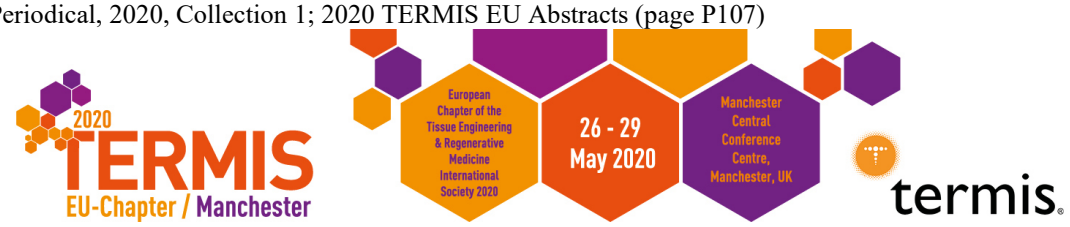
Keywords: Bone and bone disorders (osteoporosis etc), Decellularised matrices



Establishing a human ex vivo bone defect model to evaluate hydrogels or solid biomaterials for bone regeneration

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INTRODUCTION:Testing of new implant materials and bone repair strategies rely mainly on in vivo and in vitro investigation models providing different pros and cons. In vitro models provide the advantage of highly controllable test conditions enabling to assess the detailed mechanistic impact of materials, growth factors or cells on the bone regeneration process, while in vivo models are widely considered to be more suitable to predict the overall clinical outcome. In this study we established a novel human ex vivo bone defect model with a proven vitality of at least 28 days. The model provides a native bone implant interface and is designed to monitor cell invasion into a critically sized defect filled with the potential implant material.

METHODS:Femoral heads derived from patients undergoing hip replacement were cut into cylinders (20 mm diameter, 7 mm height). This size can be adapted depending on the application. For all set ups a central bone defect (6 mm diameter, 5 mm depth) was inserted centrally.
The bone slides were cultured for 28 days and viability was evaluated by lactate dehydrogenase and alkaline phosphatase assay, as well as Calcein-AM staining and DNA quantification. To analyze the bone regeneration potential in this model, we injected collagen-type 1 and Gelatin-methacryloyl (GelMA) hydrogels into the central defect or applied commercially available Maxcraft ®. Ingrowth of cells was investigated via Confocal microscopy, DNA quantification and quantitative real time PCR.

RESULTS:Data revealed the viability of the bone tissue over the tested time period of 28 days, as well as an increase in cell numbers in the bone tissue implicating active cell proliferation in the bone. Cellular ingrowth into the collagengels was evaluated by microscopy and DNA quantification at different time points demonstrating an increase of cells over time. Finally, gene expression of osteogenic markers indicated an osteoblastic phenotype of the cells in the defect1. Initial results for additional implant materials such as GelMa hydrogels with different methacrylation degrees or commercially available bone materials such as Maxcraft ® also indicated an active regeneration process at the bone implant interface to be further refined.

DISCUSSION & CONCLUSIONS:In summary, the ex vivo bone defect model remains viable and shows active bone repair processes over 28 days, monitored and quantified using cell- and molecular biological methods. Further, the processes at the native bone-implant interface can be documented in the context of different implant materials.

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Keywords: Interfaces - biological, Trauma / surgery and rehabilitation

The effect of interleukin 1 beta on osteochondral tissue in a novel patellar explant model of osteoarthritis

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INTRODUCTION:Post-traumatic osteoarthritis (PTOA) occurs secondary to acute joint injury such as anterior cruciate ligament (ACL) rupture. ACL injuries are often accompanied by subchondral bone damage, which is seen by Magnetic Resonance Imaging (MRI) in the form of Bone Marrow Lesions (BML) in 80% of patients[1]. Hence, in this project we developed a novel osteochondral explant model to study subchondral bone damage in the degradation of cartilage via bone-cartilage crosstalk. In a pilot study, the inflammatory mediator interleukin 1 beta (IL-1β) was used as a surrogate for mechanical bone damage. The primary outcome was to study the catabolic markers of bone and cartilage which play an important role in the initial stages of the disease.

METHODS:Patellae were harvested from sprague dawley rats (n=48) and maintained in high glucose media with or without IL-1β. Metabolic viability was evaluated using Live/Dead and Alamar Blue assays. Glycosaminoglycan and sulfated glycosaminoglycan content were used to assess cartilage status, and alkaline phosphatase to measure osteoblastic activity. Immunohistochemistry staining against matrix metalloproteinase 13 (MMP13) developed with DAB-peroxidase (vector lab) as a chromogen and haematoxylin used as a nuclear counterstain.

RESULTS:Results showed that both proteoglycan content and bone metabolism markers were significantly increased ($p<0.05$) after 7 days in culture following treatment with IL-1β. This was further confirmed with the MMP13 immunofluorescence staining which showed an increase in the expression of this marker following one day of IL-1β treatment. Qualitative morphological observations from these studies also suggest that chondrocytes may undergo apoptosis following prolonged IL-1β treatment.

DISCUSSION & CONCLUSIONS:To confirm our hypothesis caspase-3 staining would be required. Therefore, both quantitative and qualitative studies demonstrate that the patellar explant model can be a useful model to study the role of bone damage in PTOA via bone-cartilage crosstalk.

Acknowledgements:Science Foundation Ireland, (17/CDA/4699)

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Keywords: Bone and bone disorders (osteoporosis etc), Cartilage / joint and arthritic conditions

3D triple culture of primary human osteocytes, osteoclasts and osteoblasts – an in vitro bone model to evaluate the influence of bioactive molecules and biomaterials on bone metabolism

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INTRODUCTION:Bone tissue is a complex system of different, specialized cell types, spatially organized in a mineralized extracellular matrix. To investigate the cellular interaction and signaling between the major three bone cell types, osteoblasts, osteocytes and osteoclasts, an in vitro tripel culture model would be advantageous, opening the opportunity to analyze the cell types separately and to investigate the influence of biomaterials, drugs and bioactive molecules on the cross talk between bone cells. In the present study exclusively human primary osteoblasts, osteoclasts and osteocytes were spatially arranged in 3D collagen constructs to form the first completely human in vitro bone model.

METHODS:Human osteocytes were obtained after two-step differentiation of primary human osteoblasts, isolated from femoral heads after total hip replacement surgery [1]. Human osteoblasts and mature human osteoclasts [2] were seeded on a porous membrane in close contact to the collagen gel, which contained embedded osteocytes. Seeding was performed with the help of a 3D printed silicon mask to achieve a pattern of osteoblasts and osteoclasts allowing the separate analysis of these two cell types without preventing direct cell-cell contact. After 7 days of co-cultivation triple and single cultures were subjected to fluorescence microscopy, gene expression analysis and analysis of osteoblast, osteoclast and osteocyte markers on protein level.

RESULTS:Osteoblasts, osteocytes and osteoclasts retained their specific morphology in the triple culture and expressed their specific marker genes: tartrate resistant acid phosphatase and cathepsin K for osteoclasts, alkaline phosphatase, bone sialoprotein II and osteocalcin for osteoblasts and E11, sclerostin and osteocalcin for osteocytes. Furthermore, alkaline phosphatase activity of osteoblasts as well as the activity of osteoclast specific enzymes was proven. Osteocalcin, which is a late osteoblast and early osteocyte marker, was differentially regulated in the triple cultures: Its gene expression was significantly downregulated in tripel-cultured osteocytes compared to single osteocyte cultures. In contrast, osteocalcin expression of osteoblasts was significantly upregulated in triple culture.

DISCUSSION & CONCLUSIONS:Tripel cultures from primary human osteoblasts, osteocytes and osteoclasts were successfully established in collagen gel constructs. The problem of separate analysis of the three cell types was solved by 1) separating osteocytes from the other two cell types by a porous membrane and 2) patterned seeding of osteoblasts and mature osteoclasts.

Acknowledgements:This work was financially supported by the Deutsche Forschungsgemeinschaft (DFG) (BE 5139/3-1).

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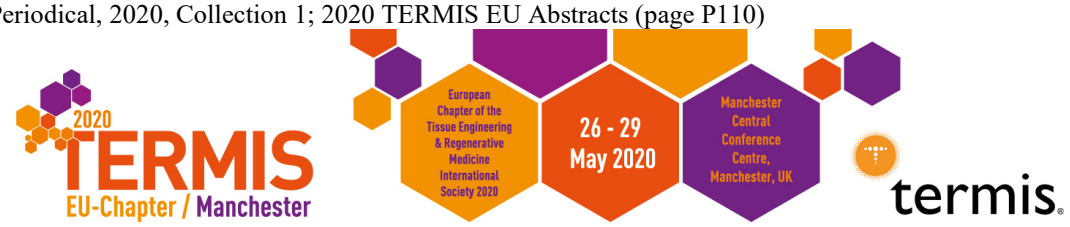
Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Differentiation



Analyses of periosteal cell-seeded β-tricalcium phosphate constructs by Raman spectroscopy

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INTRODUCTION:Non-invasive technologies for live-monitoring of the maturity level of future three-dimensional (3D) tissue engineering constructs have to be adapted and optimized. In our previous studies, we performed quality analysis of jaw periosteal cells (JPC) formed extracellular matrix (ECM) in two-dimensional cell cultures either under cultivation in FCS-free media or supplemented with FCS or human platelet lysate (hPL) by Raman spectroscopy. In the present study, we performed Raman analyses of 3D-cultivated JPCs grown within β-tricalcium phosphate (β-TCP) constructs.

METHODS:Human JPCs were grown within β-tricalcium phosphate (β-TCP) as well as within polylactic acid coated (β-TCP/PDL) constructs for 17 days under undifferentiated and osteogenic conditions. In order to determine the compressive strength (N/mm2) of the uncoated/coated β-TCP biomaterial, 8 measurements per scaffold type were undertaken before cell colonialization.
Raman spectra of 3D-cultivated JPCs from 3 donors were recorded using a Renishaw InVia Microscop Qontor Raman device.
Cell-seeded constructs were fixed, sawn and sanded after embedding in technovit 8100. Sections were stained by Levai-Laczko.

RESULTS:The mechanical tests showed significantly higher compressive strength of coated β-TCP constructs in comparison to that of uncoated core material. Histological analyses showed a relatively homogenous cell colonialization of both materials. On the surface of the composite material, a thin but very dense cell layer was detected, whereas the uncoated β-TCP core material seemed to be covered by a thicker layer of loosely distributed cells.
The recorded Raman spectra from cell-free scaffolds showed clear differences in dependence of the material and the culture conditions. The composite material could be clearly identified by a specific peak at 874 cm-1. As expected, on the surface of uncoated scaffolds, the amount of free phosphate groups was shown to be much higher than on the surface of PDL-coated scaffolds.
The recorded Raman spectra of cell-seeded scaffolds revealed in general much lower signal intensities. Due to strong scattering of the material, the cell-specific hydroxyapatite peak was covered by material-specific peaks. However, cell material could be clearly identified by two specific peaks (phenylalanine at 1004 cm-1 and coenzym A signal at 1557 cm-1).

DISCUSSION & CONCLUSIONS:Obtained data from this study showed clear differences between composite and uncoated β-TCP material as well as between cell-seeded and cell-free constructs. However, the cell-specific hydroxyapatite peak is difficult to be identified due to strong scattering of material. In the next step, data analysis has to be optimized in order to be able to detect cell mineralization within ceramic scaffolds.

Keywords: Biomaterials, Stem cells – general

Polyphenols recovered from waste of olive oil production as bioactive compounds in bone regeneration

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INTRODUCTION:The olive oil industry produces large amounts of waste such as olive mill wasterwater a sediment deposited on the bottom of unfiltered Extra Virgin Olive Oils (EVOOs). This waste is usually disposed-off with a relative cost for the companies [1]. Among all the industrial waste there are many polyphenols compounds that are unfortunately lost during the technological processes, while natural method leaves these substances within the olive oil [2]. Polyphenols are widely studied for their antioxidant activity and are therapeutically exploited as foods, food supplements or dermatological products. In bone tissue regeneration, many studies evidence polyphenols' activity on osteoblast and osteoclast, in particular Hydroxytyrosol (HT) [3]. With the support of Cariplo Foundation we studied the possibility to use waste products from olive oil production in bone regeneration with polyphenols enriched scaffolds.

METHODS:After EVOO sediments collection in olive mills, located in the Campania region (Italy), polyphenols were extracted [4] and analyzed. RP-HPLC was used to quantify HT in the extracts. 3D scaffolds in collagen, alginate or polylactic acid (PLLA) were prepared in sterile conditions and functionalized with HT at different concentration. To verify the release of HT from the scaffolds, samples were incubated in physiological conditions and analyzed by RP-HPLC at different times. In vitro tests were done using human primary osteoblasts (hOB) in order to evaluate the effect of the medicated scaffolds on proliferation (MTT test) and differentiation (ALP and mineralization tests).

RESULTS:The total phenolic content of the EVOO sediments varies from different regional locations. The HT added to the scaffolds is active on proliferation and differentiation of hOB. Preliminary test showed no HT cytotoxicity at the concentrations selected.

DISCUSSION & CONCLUSIONS:Our preliminary data show that this waste can be considered as a novel source for the extraction of phenolic compounds that, added to the 3D bioactive scaffold, can be released in the biological environment with osteoconductive activity and without cytotoxicity. The preliminary data obtained are promising in view of a medicated scaffold that will speed bone tissue regeneration.

ACKNOWLEDGEMENTS:This work was supported by Fondazione Cariplo who funded the project in the framework of the call "Economia Circolare-Ricerca per un Futuro Sostenibile" (grant agreement 2018-1001).

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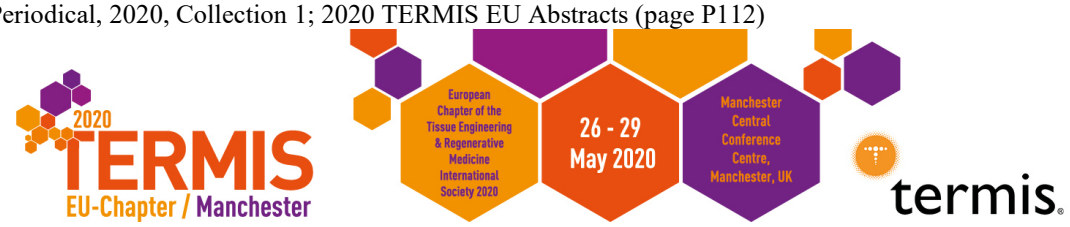
[4] Del Monaco G et al. Food Chem. 2015, 184:220-228.

Keywords: Bone and bone disorders (osteoporosis etc), Advanced therapy medicinal products



Perfusion Bioreactor for constructing a dynamic in vitro model of Ovarian Cancer

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INTRODUCTION:Ovarian Cancer (OC) research is limited by the lack of an appropriate in vitro model of the tumor microenvironment. We utilized a perfusion bioreactor, suitable for culture of up to 40 cell-seeded scaffolds (1), at velocities mimicking the cellular conditions in the extracellular fluid flow, to construct a new OC model. Our hypothesis was that operating the reactor at low volumetric velocities, with vertical upward flow, will allow better homogeneous cultivation, compared to horizontal fluid, highly affected by gravitation forces.

METHODS:Velocities and shear stresses throughout the reactor body were simulated by ANSYS Fluent, at different velocities, of vertical or horizontal flow. ES2 and 433 OC cells were seeded into alginate macroporous scaffolds and cultured in the perfusion bioreactor for 3 days under the simulated conditions. Cell viability was examined by Presto Blue assay. RT-PCR analysis was conducted for Spingosine-1-Phosphate receptors (S1PRs), associated with OC. The mRNA expression levels, and their relative proportions were compared to other culture methods (monolayer, static seeded scaffolds, spheroids) and to OC samples of primary tumor and effusions.

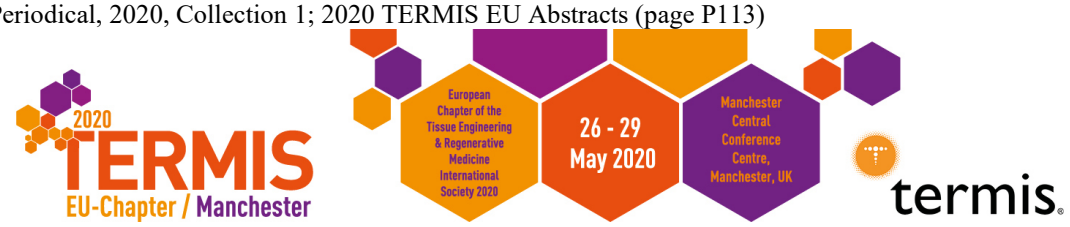
RESULTS:ANSYS Fluent simulation indicated higher homogeneity at 50mL/h vertical flow, compared to horizontal flow conditions. ES-2 seeded scaffolds culture at 50 mL/h vertical flow, resulted in more homogenous cellular viability, compared to horizontal flow; supporting the simulation results. mRNA expression levels of S1PR1 and S1PR2 were shown to be significantly lower when 500 mL/h volumetric velocity was applied, compared to 50 mL/h and static conditions ($p<0.05$); emphasizing the necessity of specific velocity for the OC in vitro model.
433-seeded scaffolds, cultured at 50 mL/h resulted in S1P receptor mRNA expression levels, similar to those of the primary OC samples, compared to monolayer, static scaffold and spheroid cultures.

DISCUSSION & CONCLUSIONS:We have developed a perfusion reactor system that at vertical upward flow and specific flow velocity mimicked the solid ovarian carcinoma parameters in vivo.

Acknowledgements:Azrieli College of Engineering, Jerusalem (AB)

References:1. Dvir T, Benishti N, Shachar M, Cohen S. A novel perfusion bioreactor providing a homogeneous milieu for tissue regeneration. Tissue Eng. 2006;12(10):2843–52.

Keywords: Cancer, Bioreactors



Role of fiber thickness and surface treatment of electrospun polycaprolactone scaffolds on the growth of different breast cancer-associated cells

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INTRODUCTION:Personalized cancer models are necessary to successfully translate oncological drugs and predict patient-specific therapeutic responses. In vivo models could be cell line-derived xenografts (CDX) composed of cell lines such as MDA-MA-231, or patient-derived xenografts (PDX) composed of primary patient-derived tumor cells. In this study, we tested electrospun poly-caprolactone (PCL) scaffolds towards the development of an optimal in vitro and in vivo breast cancer model. We evaluated the effects of fiber thickness and surface treatment of electrospun PCL scaffolds on in-vitro growth of three breast cancer-associated cell types MBA-MB-231, adipose-derived stem cells (ADSC) and endothelial progenitor cells (EPC).

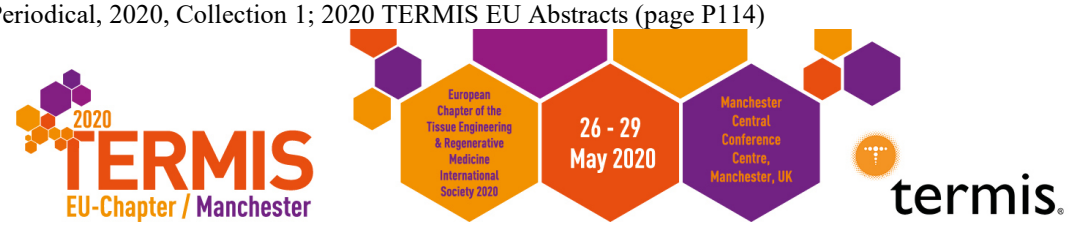
METHODS:PCL was electrospun in random-orientation into nanofibrous, micro/nanofibrous, and microfibrous scaffolds and in an aligned-orientation. Fiber diameters were measured by scanning electron microscopy (SEM). Surface-treatment process with air-plasma was characterized by X-ray photoelectron spectroscopy (XPS), water contact angle (WCA) measurements. Cell proliferation of three breast cancer cell types (MDA-MB-231, ADSC, EPC) was measured over 12 days by WST-8 assay. The cellular infiltration within the scaffolds was analyzed microscopically by cross-sectioning the scaffolds after DAPI (4′,6-diamidino-2-phenylindole) and actin staining.

RESULTS:Fiber diameters were the following: random-oriented nanofibrous: 125 ± 32 nm, micro/nano fibers: 1570 ± 383 nm / 462 ± 173 nm, microfibers: 8801 ± 716 nm; aligned fibers: 865 ± 104 nm. Air-plasma treatment reduced the WCA, while XPS analysis revealed a decrease in carbon ratio and an increase in oxygen and nitrogen ratios. On all scaffolds cells proliferated over time. EPCs proliferated significantly better on scaffolds after plasma treatment. On nanofibrous and micro/nano fibrous scaffolds, MDA-MB-231 and EPC grew on the surface. In contrast, microfibers allowed cellular infiltration into the scaffolds. Proliferation of MDA-MB-231 and EPC was similar on all scaffolds, while ADSC showed significantly higher proliferation on nanofibers. Aligned matrices did not lead to any significant difference in the proliferation rate of MDA-MB-231 cells than that on random matrices.

DISCUSSION & CONCLUSIONS:Although nanofibers show better proliferation of ADSC, microfibers have better cellular infiltration of all the three cell types. Air-plasma treatment resulted in increased hydrophilicity of scaffolds, which is beneficial for the growth of EPC. Although further functional assays are needed for defining an optimized scaffold, our findings suggest that plasma-treated electrospun PCL scaffolds can be a powerful tool to design a 3D-breast cancer model containing different cell types.

ACKNOWLEDGEMENTS:Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 326998133 – TRR 225 (subprojects B01,B06, C03), and the DAAD (Programme ID: 57440921).

Keywords: Disease models, In vitro microenvironments



Measuring tumor protrusion fluctuations as a guide to assess their metastatic potential

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INTRODUCTION:Recent evidences have shown that the tumor cells modulate protrusions phenotype and dynamics to optimize their invasion capacity in 3D microenvironments [1]. However, the role and function of protrusion morphodynamics at earlier stages of tumorigenesis are still not well understood [2]. Herein, we analyze their role in vitro and define novel parameters of tumor invasiveness.

METHODS:Human MCF7/MCF10A breast cancer/healthy cells and A549 lung adenocarcinoma cells (ATCC) were encapsulated for 3 days into a collagen I 3D matrix at low density to form small spheroids. For drug experiments, the cells were incubated with 0.1, 1.0 and 10 ug/mL of doxorubicin, 1 ug/mL C3 Rho inhibitor, and 50 nM TGF-b1. Tumor protrusion fluctuations were monitored by time-lapse. Biophysical parameters: frequency of probing (fp), and stabilization lifetime (ts) were measured.

RESULTS:Protrusion fluctuations displayed a distinct morphodynamics depending on tumor metastatic capability. Non-metastatic A549 spheroids showed a lower fp and larger ts compared to more-metastatic MCF7 and healthy MCF10A. The addition of DOX and TGF-b1 perturbed protrusion fluctuations, increasing (decreasing) fp (ts) in both cell types, resulting in an enhanced tumor invasiveness. Interestingly, we found a linear correlation between both the parameters, which correlated with the invasive potential of the cells. We also found that Rho inhibition modulated fp and ts, which abolished the invasive capacity of tumors. Next, a tumor-on-chip was employed to integrate our spheroids and endothelial cells mimicking the native scenario. The endothelial cells affected protrusion fluctuations, suggesting a crosstalk between both cell types. Finally, all these observations were encoded into a phase diagram, which provided a novel landscape capable to assess the invasiveness of tumors based on their tumor fluctuations.

DISCUSSION & CONCLUSIONS:The data illustrate that protrusion fluctuations are key players in the physicochemical mechanism of tumor invasion, and are governed by fp and ts.

ACKNOWLEDGEMENTS:Portuguese FCT (CEECIND/00352/2017, PTDC/BTM-ORG/28070/2017, PTDC/BTMORG/28168/2017); H2020 EU (FoReCaST-668983)

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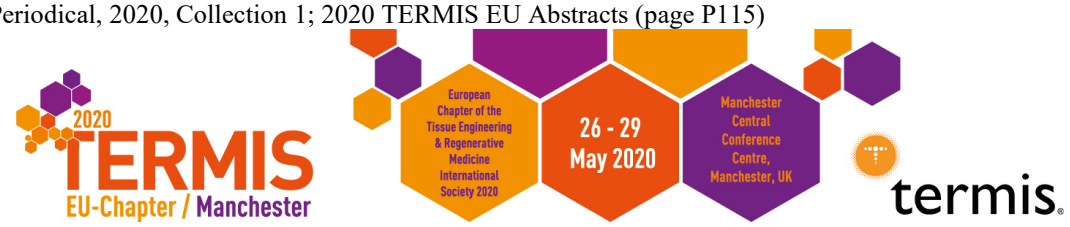
Keywords: Cancer, Biomechanics / biophysical stimuli and mechanotransduction



Human immuno-competent organotypic model of Malignant Melanoma: An innovative in-vitro tool for the preclinical efficacy assessment of immunotherapies

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INTRODUCTION:As Malignant Melanoma (MM) cells evade from the immune system through various mechanisms including the activation of immune checkpoint pathways, immune checkpoint inhibitors (ICI) were the first class of therapy shown to improve the overall survival in patients. Only a small subset of melanoma patients respond to ICI. Consequently, new immunotherapies are urgently needed. However, preclinical development of immunotherapeutics depends on animal experimentation as classical cell culture has failed so far to predict clinical outcome. Our aim is to extend an organotypic melanoma model generated using human skin cells and various melanoma cell lines by the addition of human T cells. These human-based immuno-competent organotypic models will be applied as a novel and innovative in-vitro platform for the preclinical evaluation of immunotherapies.

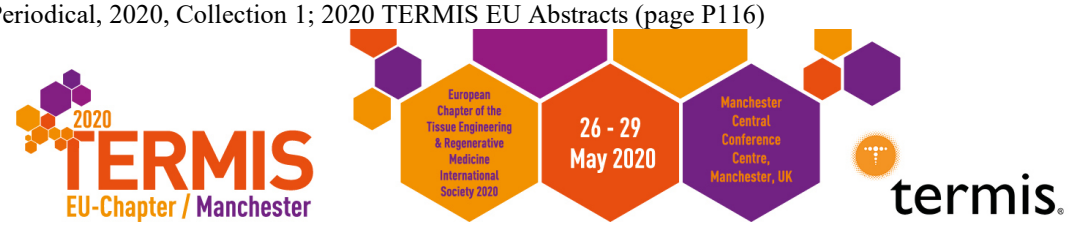
METHODS:Reconstituted human epidermis (RHE) as well as full thickness skin equivalents (FTSE) of a melanoma phenotype of various developmental stages were established using different melanoma cell lines characterized beforehand. RHE were generated adapted to the refined open source reconstructed epidermis (OS-REp) protocol (Groeber et al., 2016): primary juvenile human epidermal keratinocytes (hEKs) and melanoma cells were mixed in a defined ratio and cultured at Air Liquid Interface (ALI). In contrast, FTSE obtain in addition a dermal compartment consisting of human dermal fibroblasts (hDF) embedded in Collagen (Col) I gel. To reflect late stages of tumor formation melanoma cell spheroids were mixed with hEKs and applied to generate RHE or FTSE. First Co-culture models were established by adding human T cells isolated from peripheral blood mononuclear cells (PBMCs) to the skin models.

RESULTS:We succeeded to create organotypic melanoma models of various complexity and different tumor stages reflecting not only the physiological but also correlating with the in-vivo situation. The histological structure as assessed by immunohistochemistry as well as expression of specific melanoma markers analyzed using immunocytochemistry revealed that the formed melanoma could be detected physiologically located at the basal layer of the epidermis. First immuno-competent models showed the expression of specific markers whereas T cells assessed by immunohistochemistry could be detected within the model.

DISCUSSION & CONCLUSIONS:We established a first human-based immuno-competent organotypic model of MM which display an initiative step towards a powerful in-vitro tool for the preclinical evaluation of the efficacy of immunotherapeutics. Moreover, the successfully created physiological organotypic model of MM offers the possibility to address all kinds of scientific questions regarding for example tumor growth, tumor progression or cell-cell interaction.

Acknowledgements:This project is funded by BMBF.

Keywords: Disease models, Immunity / immunomodulation / macrophage



Key regulator of pancreatic development PDX1 and its anti-metastatic function in pancreatic ductal adenocarcinoma

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INTRODUCTION:Pancreatic ductal adenocarcinoma (PDAC) is on the way from the fourth to the second top position in global cancer mortality by 2030. The frequency of emergence and mortality of PDAC is almost the same due to the combination of usually late diagnostics, aggressive clinical course, and poor reaction to chemo- and radiotherapy. In PDAC, as well as in other malignant tumors, metastasis is the main cause of death of cancer patients and often occurs at early stages of cancer development. In cancer biology, metastasizing is one of the most poorly studied phenomena. Recently, the attention of researchers was attracted by PDX1, the key regulator of the pancreas development, as possible factor involving in suppression of spreading cancer cells. This work is devoted to the study of the role of the PDX1 gene in the progression of PDAC.

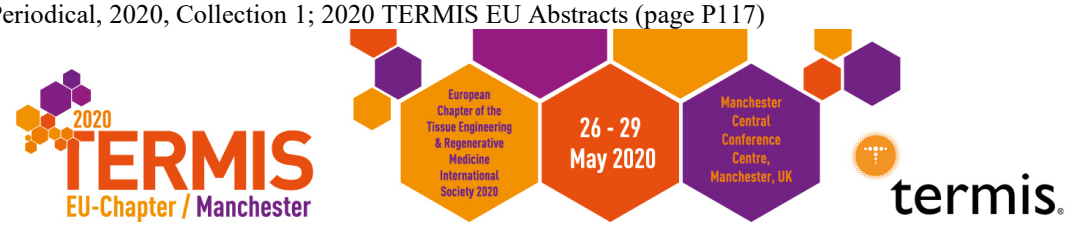
METHODS:The expression level of PDX1 in the tumor samples of this human organ was determined by Real-time PCR. To study the effect of PDX1 overexpression on the malignant potential of cancer cells, transduced PANC-1 and Colo357 lines expressing PDX1 and control cells were obtained. Effects of PDX1 ectopic expression on pancreatic cancer proliferation and motility were determined in PANC-1 and Colo357 cells using MTS, cell cycle analysis, transwell and wound-healing assay. Assessment of the migration potential of pancreatic cancer cells expressing PDX1 was evaluated in zebrafish model.

RESULTS:The expression level of PDX1 in 22 tumor samples was 10 times ($p < 0.001$) less than in the normal pancreas, and in 17 tumor samples it was comparable to the normal level. In model 6 PDAC cell lines, PDX1 expression is absent. It was shown that PDX1-positive PANC-1 and Colo357 cells possess increased growth rate of 1.7 ($p < 0.01$) and 1.6 times ($p < 0.05$), respectively. Migration through Transwell and the rate of wound healing in PANC-1 and Colo357 cells expressing PDX1 were reduced by 50% compared to control cells ($p < 0.01$). In a two-day D.rerio embryo model it was shown that PDX1-positive PANC-1 cells exhibited a lower level (averagely 12.5%) of migration as compared with control PANC-1 cells (averagely 50%). Suppression of PDX1 expression by siRNA restores motility.

DISCUSSION & CONCLUSIONS:Our in vitro and in vivo results may indicate that expression of the PDX1 gene inhibits metastasis of cancer cells.

ACKNOWLEDGEMENTS:The study was supported by the Russian Science Foundation (project no.14-50-00131).

Keywords: Disease models, In vivo and animal models



Investigating the molecular identity of cardiac progenitor cells for applications in heart disease

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INTRODUCTION:With an increasing incidence, compounded by the heart's inherent lack of regenerative capacity, heart disease is a growing burden on health care and a significant cause of death. Human pluripotent stem cell (hPSC)-derived cardiac progenitor cells (CPCs) have therapeutic promise for myocardial regeneration, but their molecular identity remains ill-defined and the conditions required for CPC self-renewal and differentiation are poorly understood. The drug regulatable transgene system and fluorescent reporter in the Tet-On-MYC NKX2-5eGFP hPSC line[1] enables expansion of CPCs which can then be specified to express the cardiac marker NKX2-5, as visualised by GFP. These CPCs can be further differentiated to cardiomyocytes and endothelial cells. The differentiation potential of progenitor cells depends on their earlier lineage specification, however in heart development this relationship is poorly understood.

METHODS:To explore how developmental patterning impacts progenitor (including CPC) fate, I varied the signalling gradients of BMP4 and Activin A, mimicking gastrulation-like events that occur during development, to direct differentiation of hPSCs towards CPC, anterior mesoderm-like and posterior mesoderm-like populations. From this range of populations, clonal spheres were isolated, tested for NKX2-5 induction capacity and terminally differentiated.

RESULTS:My data so far confirm that clonal fate can be quantified, varies by patterning with BMP4 and Activin A and is as expected from studies of developmental biology. Using RNA-seq analysis, I am currently examining gene expression differences in progenitor populations that vary in NKX2-5 induction capacity and potential for differentiation to cardiomyocytes and endothelial cells.

DISCUSSION & CONCLUSIONS:By identifying the genes which functionally determine their fate potential, these data will greatly improve our understanding of the molecular identity of CPCs. These advances are an important step towards using CPCs in a therapeutic context.

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Keywords: Embryonic stem cells, Developmental biology

Copper-silk sericin hybrid as pro-angiogenic therapeutic for ischemic treatment

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INTRODUCTION:Angiogenesis, the process of formation of new blood vessels, plays critical role in physiological (e.g. embryonic development) and pathological conditions (e.g. ischemic disease, wound healing, cancer). Ischemic heart disease (IHD) is one of the leading causes of death worldwide [1]. Therapeutic angiogenesis aims to treat IHD patients, specifically who are unable to undergo bypass surgery or angioplasty. Pro-angiogenic therapeutic agents with anti-inflammatory effects are considered as potential therapeutic candidates in IHD treatment. Copper is known to trigger angiogenesis in endothelial cells. Silk sericin of silkworm ""Bombyx mori"" is a hydrophilic, glue-like protein with anti-inflammatory property [2]. Hence, the angiogenic and inflammatory responses of silk sericin-copper hybrids are evaluated hereby, envisioning the hybrid as effective therapeutic candidate for treating IHD.

METHODS:The copper-silk sericin hybrid was obtained by a one-pot fabrication method using aqueous copper sulfate solution and silk sericin prepared in phosphate buffer saline (pH 7.4) at room temperature. Sky blue colored precipitation of Cu-sericin hybrids was collected by centrifugation, washed several times and air-dried at room temperature. The in vitro angiogenic potential of the hybrids was investigated using human umbilical vein endothelial cells (HUVECs). To study the inflammatory response, microphages were used.

RESULTS:The protein molecule of silk sericin complexes with copper that serves as nucleation site for the copper phosphate crystal formation. The interaction between copper ions and protein results hybrid micro-particles, which can sustain the release of copper and sericin over time. The ""in vitro"" ell culture studies reveal higher level of angiogenesis in treated group.

DISCUSSION & CONCLUSIONS:The ""in vitro"" results indicate the potentiality of using copper – silk sericin hybrid as therapeutic candidates in IHD treatment.

ACKNOWLEDGEMENTS:This work is financially supported by the European Union Framework Programme for Research and Innovation Horizon 2020 under grant agreement nº 668983 — FoReCaST; FROnTHERA (NORTE-01-0145-FEDER-000023); Investigator FCT program (IF/01214/2014) to VMC and Investigator FCT2015 (IF/01285/2015) to JMO.

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Keywords: Composite materials, Nanomaterials (inc graphene)

Scaffold morphology and ECM composition: key factors in vascular tissue engineering

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INTRODUCTION:There are currently a variety of vascular tissue engineering approaches used to treat arterial disease1. These range from scaffold materials that mimic the native extracellular matrix (ECM) to materials designed to promote angiogenesis2,3. However, native autologous vessels used as implants still outperform these novel materials4. Therefore, finding solutions that promote vascular regeneration is of crucial importance for the advancement of this field. Herein, we have looked at how the fiber diameter and protein composition of electrospun polymer scaffolds affects human umbilical vein endothelial cells (ECs) and human umbilical aorta smooth muscle cells (SMCs) performance.

METHODS:Scaffolds were electrospun using varying electrospinning parameters in order to achieve fibers of different diameters. Briefly, 8% and 12% w/v polycaprolactone (PCL) solutions in HFIP; and 14% and 19% w/v PCL solutions in 5:1 chloroform:methanol were electrospun at 250 RPM mandrel speed, resulting in four randomly aligned PCL scaffolds with different fiber diameters. Scaffold composition was further optimized through the addition of decellularized vascular ECMs. Scaffolds were either seeded with ECs or SMCs. Various quantification methods were performed at time points of 1 day, 6 days and 12 days, including cell viability, RT-qPCR and mechanical analysis, amongst others.

RESULTS:Fiber diameter and pore size analysis showed that four unique architectures were created, with incrementally increasing diameters/widths ranging from approximately 1.5µm to 5µm. We noted increased cellular infiltration in the largest fibre scaffold. Decellularized ECM was successfully incorporated into the scaffold and confirmed using Fourier Transform Infrared analysis. Furthermore, interesting trends in gene expression were noted suggesting that altering the morphology of the scaffold had influences on the seeded cells.

DISCUSSION & CONCLUSIONS:This systematic study has shown that cellular performance and gene expression can be modulated through altering the fibre diameter and protein composition of the scaffold. We found increase infiltration with the largest fibre, which lead to positive changes in gene expression.

ACKNOWLEDGEMENTS:We would like to acknowledge the following funding ESPRC no. EP/N509644/1 and MRC grant MR/L012766/1

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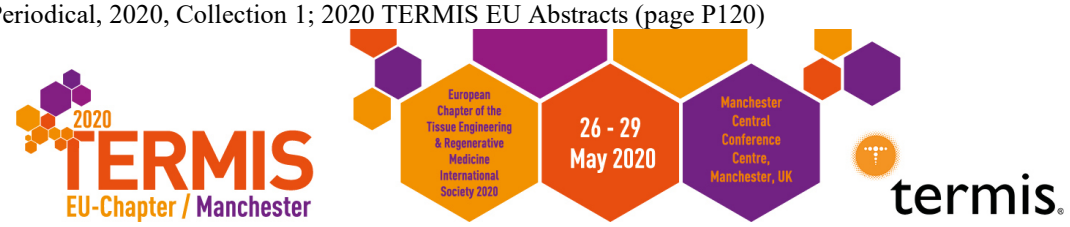
Keywords: Vascular systems / vascularisation and heart, Biomaterials



The role of inflammation on chondrocyte hypertrophy and its potential implications in regenerative medicine

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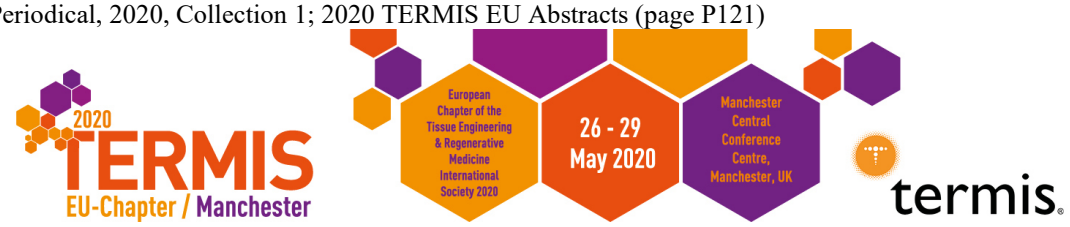
INTRODUCTION:Regeneration of articular cartilage is dampened by the inflammatory microenvironment in the joint. Moreover, inflammation induces hypertrophy of mouse chondrocytes, which leads to permanent cartilage changes. Whether inflammation also stimulates hypertrophy in primary human chondrocytes remains unclear. We therefore investigated whether pro-inflammatory cytokines or medium conditioned by macrophages stimulates this hypertrophic shift in human chondrocytes.

METHODS:Cartilage explants and chondrocytes were isolated from donors who underwent total knee arthroplasty. Chondrocytes were encapsulated in alginate beads. IL1-β, TNF-α and IFN-γ (0.1 ng/ml) were used as pro-inflammatory stimuli and SC-514, an IKK-2 inhibitor, was used to specifically block NFκB-dependent mRNA expression. Human monocytes were cultured with IFNγ and TNFα to generate pro-inflammatory M(IFNγ+TNFα) macrophages, with IL-4 to generate tissue repair M(IL4) macrophages, or with IL-10 for anti-inflammatory M(IL10) macrophages (each stimulatory factor at 10 ng/ml). Transcript analysis on COL10A1, RUNX2 and ALPL was performed to assess chondrocyte hypertrophy, and TNFA to determine inhibition of NFκB (N=2-3 OA donors in biological triplicate).

RESULTS:Pro-inflammatory cytokines (IL1-β, TNF-α and INF-γ) decreased COL10A1 in explants and alginate-embedded chondrocyte cultures. ALPL was not detectable in either of the cultures while RUNX2 was not detectable in explants and down regulated in alginate cultures upon cytokine addition. These data indicate that exposure to inflammatory cytokines did not stimulate a hypertrophic phenotype. To evaluate whether endogenous inflammation present in osteoarthritic chondrocytes influenced hypertrophy, SC-514 was added to alginate cultures. Although this significantly decreased mRNA expression of the NFκB-dependent gene TNFA, it did neither modify COL10A1 nor RUNX2 expression.
To better mimic the joint inflammatory conditions we added macrophage conditioned medium to cartilage explants, and observed that medium conditioned by pro-inflammatory M(IFNγ+TNFα) or anti-inflammatory M(IL10) macrophages had no effect on COL10A1, RUNX2 and ALPL expression. Medium conditioned by tissue repair M(IL4) macrophages, however, significantly upregulated the expression of COL10A1 and RUNX2 in 2 of the 5 donors tested. Further analyses demonstrated that these 2 donors, differently for the other 3, were initially not hypertrophic. These data suggest that pro-inflammatory factors do not induce hypertrophic differentiation of articular chondrocytes but factors secreted by tissue repair macrophages can induce hypertrophy.

DISCUSSION & CONCLUSIONS:Chondrocyte hypertrophy was not stimulated by addition of pro inflammatory cytokines or by medium conditioned by pro-inflammatory macrophages. Moreover, inhibition of NF-κB did not inhibit hypertrophy. Conditioned medium of tissue repair macrophages, however, may contribute to the onset of this detrimental phenotype. Targeting this macrophage subset could be used to enhance articular cartilage repair.

Keywords: Differentiation,



Concentration-Dependent Effects of Cobalt and Chromium Ions on Osteoarthritic Chondrocytes

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INTRODUCTION:Cobalt (Co) and chromium (Cr) ions are released into the joint after implantation of metal implants due to biotribocorrosion. Co and Cr can induce apoptosis and alter gene expression levels in various cell types. The objective of this study was to determine the effects of Co and Cr ions on human articular chondrocytes and to evaluate inflammatory responses.

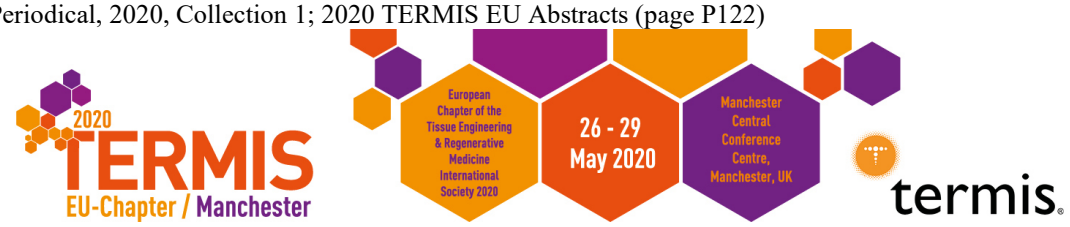
METHODS:Human articular chondrocytes were subjected to different concentrations of Co and Cr ions. Cell viability and early/late apoptosis assessment were performed in-vitro (2D cell cultures) using XTT assay and flow cytometry, respectively. Changes in chondrocytes' morphology were assessed using fluorescent cell imaging. Also, the effects on the biosynthetic activity of chondrocytes were evaluated by quantitative polymerase chain reaction (qPCR). An inflammatory response to different conditions was determined by the release of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-8).

RESULTS:Cobalt and chromium ions significantly reduce metabolic activity and induce early and late apoptosis (Annexin Vand 7-AAD) with increasing concentrations already after 24 hours in culture. After 72 hours, the majority of chondrocytes (>90%) were apoptotic. SOX 9 expression was enhanced with increasing concentrations, whereas collagen type 2 showed a linear decrease after 24 hours. IL-8 release was enhanced with increasing Cobalt and chromium ions levels, whereas IL-1β, IL-6, and TNF-α showed no significant differences between the applied conditions.

DISCUSSION & CONCLUSIONS:Co and Cr ions show a dose-dependent and time-dependent effect on articular chondrocytes. With increasing concentrations, Co and Cr ions induce apoptosis in articular chondrocytes, decrease metabolic activity and chondrocyte-specific gene expression, and induce an inflammatory response. Hence, these adverse effects on articular chondrocytes need consideration in cases of partial surface replacement and unicompartmental arthroplasty.

Acknowledgements:This work was funded by NÖ Forschungs- und Bildungsges.m.b.H. (NFB) and the provincial government of Lower Austria through the Life Science Calls (Project ID: LSC15-019) and has been carried out within Danube-University Krems – University for Continuing Education in cooperation with the Austrian Excellence Center for Tribology (AC2T research GmbH) and Landesklinikum Baden-Mödling supporting this study.

Keywords: Cartilage / joint and arthritic conditions, In vitro microenvironments



Atmospheric Pressure Plasma Facilitated Covalent Protein Immobilisation in Melt Electrowritten Fiber Scaffolds for Bioengineered Cartilage

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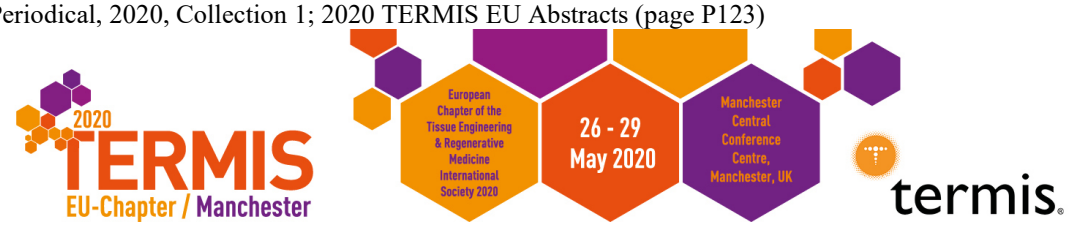
INTRODUCTION:Osteoarthritis causes disability worldwide; and currently, there is no permanent, functional cure for the joint degeneration it causes. We aim to engineer implants with both mechanical and biochemical cues that allow for functional new cartilage formation. To do this, we combine melt electrowriting (MEW) and atmospheric-pressure plasma (APP) treatment to produce polymeric fibre scaffolds with selectively and covalently immobilized growth factors (e.g. TGFβ1). We hypothesize that such scaffolds will mimic the native mechanical and biochemical cues that stimulate cells to form neocartilage.

METHODS:Poly-ε-caprolactone MEW scaffolds were fabricated using a 3DDiscovery printer (RegenHU) and then functionalized using a computer-controlled APP device (patent pending, 4.5kV discharge voltage, 1.9L/min feed gas flow, 60mm/s, 5mm spacing zigzag), generating a controlled functionalization pattern. TGFβ1 was then immobilized onto the MEW scaffold using submersion in solution (2µg/mL) (24hrs, 4°C). Detergent (Tween20 or sodium dodecyl sulfate (SDS)) washing steps were undertaken to remove non-covalently bound protein. Characterisation of immobilisation was performed by Fourier-transform infrared (FTIR) spectroscopy, immunofluorescence detection and fluorescence assays. In vitro experiments were performed by seeding equine mesenchymal stromal cells (eqMSCs) (16.6x104 cells/mL) into the MEW scaffolds and were cultured for 28 days. The culture groups consisted of (i) plasma-treated-scaffolds w/ immobilised TGFβ1, (ii) plasma-treated-scaffolds w/o TGFβ1, (iii) untreated-scaffolds w/ TGFβ1 in the culture medium, and (iv) untreated-scaffolds in basal medium. Chondrogenic differentiation was evaluated by histology and glycosaminoglycan (GAG) production.
RESULTS:Covalent immobilisation of TGFβ1 was achieved using the APP functionalization approach. FTIR confirmed the presence of a protein signature on the samples following intensive 5%SDS washing. Immunofluorescence was detected in scaffolds (following 0.1%Tween20 washing) dependent on the TGFβ1 concentration applied. In vitro analysis illustrated that GAG production (normalised to DNA) was enhanced and comparable in the immobilised TGFβ1 (i) and the TGFβ1 in medium groups (iii), compared to the control groups (ii&iv) without TGFβ1.
DISCUSSION & CONCLUSIONS:Atmospheric-pressure plasma-facilitated covalent immobilisation of TGFβ1 retains the growth factor's bioactivity and allows for cellular interactions that stimulate the differentiation of eqMSCs into the chondrogenic lineage. The ongoing work includes integration of MEW, cell deposition and plasma functionalization within a single-step printing process, allowing for real-time functionalisation during the MEW of the scaffolds for gradient and patterned effects.
Acknowledgements:This research was funded by the Netherlands Organization for Scientific Research (024.003.013), the EU's H2020 Marie Skłodowska-Curie (#801540), and an Office of Global Engagement Partnership Collaboration Award between University of Sydney, Utrecht University, and ARC.

Keywords: Additive manufacturing, Differentiation



Crosstalk in Chondrogenesis: Adipose-derived Mesenchymal Stem Cells co-cultured with articular chondrocytes from osteoarthritic patients exhibits increased chondrogenicity

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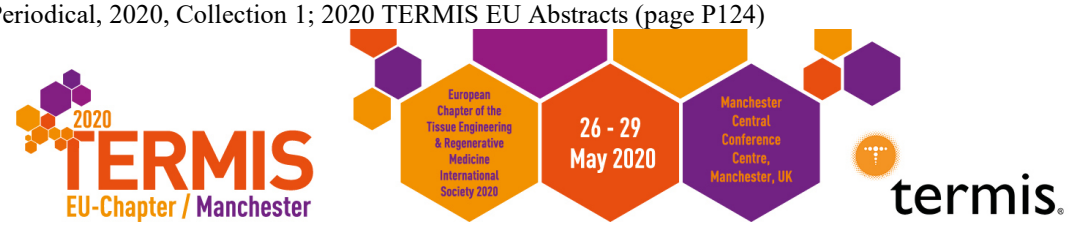
INTRODUCTION:Cell-based therapies have the potential to treat focal chondral lesions at early stages. This has the advantage of preventing the progression to more generalised osteoarthritic changes, and the ultimate need for joint arthroplasty. Although the intra-articular injection of Mesenchymal Stem Cells (MSCs) for chondral and osteochondral lesions in clinical trials has demonstrated pain reduction, the extent of cartilage repair has been variable.

METHODS:In our study we utilized an in vitro autologous co-culture of early passage (p0) adipose-derived MSCs (AD-MSCs) and articular chondrocytes derived from Grade III or IV osteoarthritic patients. The AD-MSCs were assessed using a panel of MSC cell surface marker in flow cytometric phenotyping, and allowed to differentiate down the chondrogenic, ostegenic and adipogenic pathways using established culture conditions. Gene expression following in vitro co-culture was quantified by RT-qPCR with a panel comprising COL1A1, COL2A1, COL10A1, L-SOX5, SOX6, SOX9, ACAN, HSPG2, and COMP for chondrogenesis.

RESULTS:The AD-MSCs expressed CD105, CD73, CD90, and CD34, but not CD45, CD14, CD19, and HLA-DR in flow cytometric phenotyping. The AD-MSCs also demonstrated trilineage differentiation potential. We demonstrated that chondrogenic gene expression profiles from co-cultures with juxtacrine crosstalk were greater than would be expected from an expression profile modelled on chondrocyte and AD-MSC only monocultures. Additionally, there was a decrease in chondrogenic gene expression with increasing initial MSC-to-Chondrocyte seeding ratios.

DISCUSSION & CONCLUSIONS:These findings provide insight into the mechanisms underlying clinical MSC injections and demonstrate the bidirectional MSC-chondrocyte crosstalk in inducing MSC chondrogenesis. Moreover, this signifies that pre-conditioned MSCs by chondrocyte co-culture has improved chondrogenic potential for cartilage repair. This model can be used to further understand adipose-derived MSCs in osteochondral repair and the chondrogenic pathways involved.

Keywords: Cell therapy, Multipotent (mesenchymal) stem cells



Cation releasing hydrogels for osteochondral regeneration– broadening regenerative approaches through modulating hypoxia, differentiation, and biomineralisation

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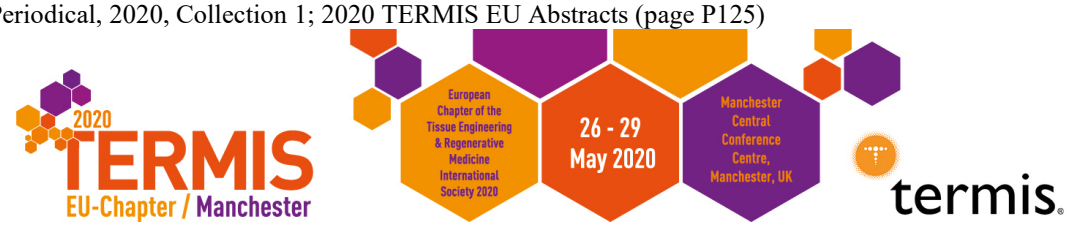
INTRODUCTION:Cartilage and osteochondral defects due to trauma or degenerative disease such as osteoarthritis represent a major clinical challenge. Associated disability, chronic pain, and morbidity limit the patients' quality of life, creating a need for novel curative approaches. Current methods to functionally restore focal defects and larger-scale damage involving the subchondral bone are limited by: limited regenerative potential of the involved tissues; patient response variability to therapeutic intervention; and failure to induce cartilage and osteochondral tissue formation following therapy. An ideal therapy should deliver the potential for functional regeneration by modulating endogenous cell behaviour without the need for cell therapy whilst employing safe, economical components amenable to accelerated translation.

METHODS:We therefore aimed to improve the regenerative potential of suitable hydrogels based on naturally occurring materials by incorporating divalent cations with different modes of action to broaden the versatility of systems for osteochondral regeneration. In this study, we augmented the activity of injectable hydrogels by incorporating cobalt or magnesium cations into 2% agarose (varying strength types) or silk hydrogels to induce hypoxia signalling and to modulate biomineralisation. Furthermore, it has been reported previously that silk fibroin protein can inherently upregulate Wnt signalling. After in-depth in vitro characterisation (release studies, biomineralisation studies, and biomechanical testing), these candidate systems were injected into acute osteochondral defects generated in the femoral groove in rat knee joints. Histological evaluation was carried out 8 weeks post-implantation (collagen I and II immunohistochemistry, trichrome, and safranin O staining). Histological scoring and qualitative evaluation were carried out to determine cartilage regeneration (O'Driscoll and Pineda) and compared to empty controls and benchmarked to a currently employed clinical scaffold candidate (Hyalofast®).

RESULTS:Whilst none of the investigated candidate systems and the clinical standard restored the cartilage phase in osteochondral defects entirely, it was observed that high-strength agarose gels out-performed the low-strength gels in partially regenerating cartilage. Furthermore, silk hydrogels were identified as best performing hydrogel matrices for regeneration alongside high-strength agarose. Supplementation of both agarose and silk hydrogels with cobalt cations increased the frequency of foci of neocartilage formation in both systems. Supplementation with magnesium did not improve chondrogenesis and resulted in formation of a fibrous repair tissue.

DISCUSSION & CONCLUSIONS:These findings indicate that the strategy of combining suitable hydrogels with cobalt-based hypoxia mimetics improves their regenerative potential. Ongoing studies include exploring further pathways for optimisation of the cobalt-containing hydrogels' chondrogenic potential by dose optimisation and fine-tuning gel stiffness parameters.

Keywords: Cartilage / joint and arthritic conditions, Hydrogels and injectable systems



Progenitors derived from healthy and osteoarthritic human cartilage show potential for cartilage tissue engineering

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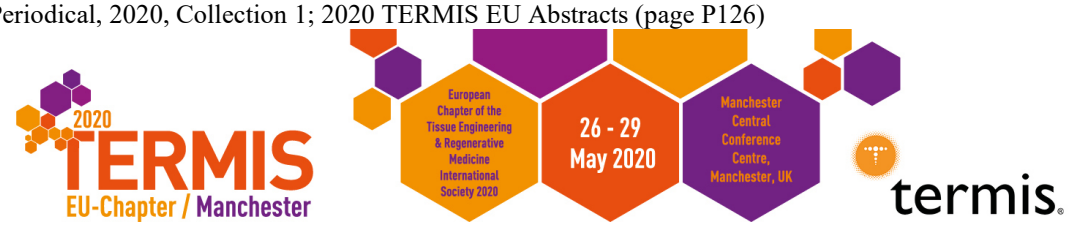
INTRODUCTION:Articular cartilage-derived progenitor cells (ACPCs) offer new interesting avenues as a cell source to use in cartilage tissue engineering. Unlike mesenchymal stromal cells (MSCs), ACPCs do not have the tendency to differentiate into the hypertrophic lineage. This study aims to isolate and characterize ACPCs from human hyaline cartilage. ACPCs from healthy and osteoarthritic cartilage were compared. In addition, the potential to use these ACPCs in co-culture with primary chondrocytes for cartilage tissue engineering is assessed.

METHODS:Cells were isolated from full-thickness healthy (n=6, age 46-49, mean age 48) and osteoarthritic (n=6, age 41-82, mean age 62) human knee cartilage. Subsequently, ACPCs were isolated from the total cell population by clonal growth after a fibronectin differential adhesion assay. MSCs were isolated from human bone marrow by plastic adherence after separating the mononuclear fraction using a Ficoll-paque density gradient. Healthy and osteoarthritic ACPCs were characterized and compared to MSCs for multilineage differentiation and via flow cytometry analysis to assess cell surface marker expression. Progenitors were also compared to full-depth chondrocytes derived from the same donors. Next, ACPCs were cultured in 3D pellets in co-culture with osteoarthritic chondrocytes and compared to co-cultures of MSCs and chondrocytes. Pellets were cultured for 28 days without the addition of growth factors. Pellets were assessed for cartilage-like matrix production using quantitative biochemical analyses for glycosaminoglycans and collagen. (Immuno)histochemistry was performed to visualize proteoglycan and collagen production.

RESULTS:Healthy and osteoarthritic ACPCs were successfully isolated and differentiated into the adipogenic and chondrogenic lineage, but failed to produce calcified matrix when exposed to osteogenic induction media. Full-depth chondrocytes derived from the same donors were able to produce calcified matrix upon induction of osteogenic differentiation. Both ACPC populations, as well as full-depth chondrocytes met the criteria for cell surface marker expression to define MSCs as determined by flow cytometry. Cartilage-like matrix production was observed in ACPC pellet cultures, as well as pellets consisting of a co-culture of ACPCs and chondrocytes.

DISCUSSION & CONCLUSIONS:In conclusion, this study provides further insight into a progenitor cell population which is present in both healthy and osteoarthritic human articular cartilage. No distinct differences were observed between healthy and osteoarthritic progenitors. The populations show similarities to MSCs, yet the progenitors did not produce calcified matrix under well-established osteogenic and mineralization culture conditions. Furthermore, ACPCs show potential for use complementary to osteoarthritic chondrocytes in cartilage tissue engineering, as a promising alternative to MSCs.

Keywords: Cartilage / joint and arthritic conditions, Stem cells – general



Characterization of chondrocytes and chondrogenic progenitor cells from auricular cartilage and perichondrium of microtia patients and healthy donors

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INTRODUCTION:Children who suffer from microtia (severe malformation of the auricle) are exposed to enormous socio-psychological distress. Tissue engineering methods could help to improve current treatment methods. Isolated chondrocytes (CC) and chondrogenic progenitor cells (CPC) from microtia patients could be a useful source for autologous in vivo cartilage regeneration, as the microtic cartilage is frequently discarded during reconstructive surgery. However, to date relatively few and inconsistent data exists with regard to biology and behaviour of CC and CPC isolated from the remnant auricular cartilage tissue of microtia patients. Therefore, this study aimed to characterize these cells and to evaluate their capability to regenerate cartilage tissue for treating microtia in more detail.

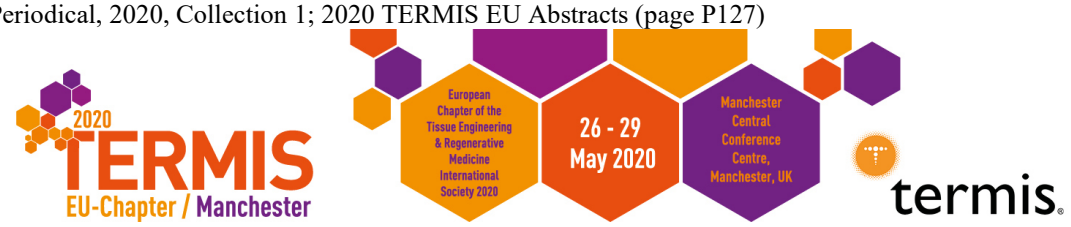
METHODS:Tissue from microtia patients and healthy donors were fixed in formalin and embedded in paraffin. Histological and immunohistochemical stainings were performed on paraffin sections for specific cartilage proteins such as collagens, aggrecans, etc to analyse the architecture of the tissues. Cells were isolated from auricular cartilage and its perichondrium of microtia patients and healthy donors using both outgrowth cell culture and enzymatic digestion. To investigate general cell properties colony forming assays and a migration assays were performed. To study cell biology, cells were cultivated in a 3D cell culture model. Spheroids were again analysed for specific cartilage tissue markers (e.g. collagen II, elastin, etc.) and specific progenitor cell markers such as Integrin β1 or Notch-1 using immunological methods (Western Blot and immunohistochemistry).

RESULTS:We observed that there was no clear boundary between perichondrium and cartilage in tissue samples from microtia patients. Regarding general cell properties, no significant differences between cells from microtia or healthy tissues were observed. CPC showed a higher capacity to form colonies and to migrate than CC. All cell types formed spheroids and expressed cartilage-like matrix. CPC isolated from the perichondrium expressed higher amounts of Notch-1 and Integrin β1 in contrast to CPC isolated from the cartilage.

DISCUSSION & CONCLUSIONS:Our results showed that CCs and CPCs isolated from microtia patients generally possess a capacity for cartilage regeneration and share many characteristics with cells derived from normal auricular cartilage. A further in depth characterization will required prior to clinical application of these cells.

ACKNOWLEDGEMENTS:Supported by the Swiss-National-Foundation (Sinergia-Project: 2-77120-17).

Keywords: Personalised medicine



Differential production of cartilage ECM in 3D agarose hydrogel constructs by ACPCs and MSCs

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INTRODUCTION:In recent years, articular cartilage progenitor cells (ACPCs) have emerged as a possible alternative to mesenchymal stem cells (MSCs) as a cell source for cartilage tissue engineering. However, they need to be characterized more extensively as only few studies have directly compared ACPCs and MSCs when cultured in hydrogels. In this in vitro study, we compared chondrogenic differentiation of equine ACPCs and MSCs in agarose constructs as monocultures and as zonally layered co-cultures under hypoxic and normoxic conditions.

METHODS:Agarose hydrogels (2%) laden with ACPCs or MSCs were cast in 55 µl silicon molds with a diameter of 6 mm. Additionally, zonally layered co-culture constructs were prepared with MSCs in the bottom layer and ACPCs in the upper layer. Constructs were cultivated for 28 days in chondrogenic medium under normoxia (21% O2) or hypoxia (2% O2). Subsequently, chondrogenic differentiation was analyzed by quantitative biochemical assays, histology, immunohistochemistry, and qPCR.

RESULTS:When each cell type was cultured alone in 3D agarose constructs, ACPCs produced significantly more glycosaminoglycans than MSCs, as determined by histological staining and quantitative assays. This was well in accordance with significantly higher gene expression for aggrecan and proteoglycan-4 (PRG4) in ACPCs, as compared to MSCs. In contrast, MSCs produced more total collagen/DNA and showed higher gene expression for type I and type II collagen than ACPCs. However, immunohistochemical staining showed that ACPCs produced less type I collagen and similar levels of type II collagen, as compared to MSCs. In co-culture, the zones of layered constructs mostly reflected the differences that were observed between ACPCs and MSCs monocultures, as shown in histological and immunohistochemical stainings. In ACPCs, hypoxia significantly increased PRG4 gene expression, but no other effects on extracellular matrix (ECM) production were observed. In MSCs, however, hypoxia reduced total collagen production and alkaline phosphatase (ALP) activity. ACPCs showed very low ALP activity in contrast to MSCs in normoxia as well as hypoxia.

DISCUSSION & CONCLUSIONS:Taken together, in 3D agarose constructs, ACPCs outperformed MSCs with regard to quantity and quality of ECM by producing tissue that was more similar to hyaline cartilage than that produced by MSCs. With this study, we provided further evidence that ACPCs represent a promising cell source for tissue engineering and cartilage regeneration.

ACKNOWLEDGEMENTS:Funding is acknowledged from EU, grant #309962 (HydroZONES), and from German Research Foundation (DFG), project number 326998133, collaborative research centre TRR225 (subproject A02).

Keywords: In vitro microenvironments, Differentiation

Potential synovial fluid biomarkers for progression of knee osteoarthritis using a multiplex ELISA based approach

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INTRODUCTION:In pathogenesis of osteoarthritis (OA), the inflammation is playing a pivotal role. Therefore, understanding the profile of inflammation in OA patient population is an essential starting point to predict or prevent OA progression. The synovial fluid (SF) is the most applicable bio-fluid to investigate progression of OA, because of its direct and intimate relationship with different tissues of knee joint. The aim of this study was to identify the profile of selected biomarkers in SF and investigate the correlation according to gender, age and severity of the disease within patients from among the general knee OA population.

METHODS:The concentration of interleukins (IL-6, IL-8), matrix metalloproteinases (MMP-1, MMP-3, MMP-13), MMPs inhibitors (TIMP-1, TIMP-2), COMP and adipokine (adiponectin) was measured and analysed in SF using a multiplex ELISA based approach from 65 OA patients (46 patients with early OA and 19 patients with end-stage of OA according to the Kellgren and Lawrence grading scale).

RESULTS:The concentration level of analysed biomarkers was not significantly different according to gender, except of adiponectin (Median: 4753 ng/mL vs. 1325 ng/mL, P=0,0289). The levels of MMP-13 and COMP protein in synovial fluid were positively correlated with the age of patients ($r = 0.2813$, $p = 0,0295$ and $r = 0.3684$, $p = 0,0164$, respectively). We detected no significant difference of IL-6 and IL-8 concentration in early OA SF compared with end-stage OA synovial fluid. Nor were any remarkable differences found in concentration levels of these cytokines according to age or gender. Significant differences in the concentration of MMP-3, TIMP-1 and COMP (25054 pg/mL vs. 35705 pg/mL, 12955 pg/mL vs. 15734 pg/mL, 3324 pg/mL vs. 4601 pg/mL, respectively) were detected in the SF of patients with early OA compared to the group of patients with end-stage OA.

DISCUSSION & CONCLUSIONS:To our knowledge, this is the first study in a Slovak population that compares and correlates a profile of synovial inflammatory mediators in SF from OA knee with other important variables such as age, sex and severity of disease. Furthermore, three biomolecules (TIMP-1, MMP-3 and COMP) were identified, that correlate with OA grade and could have the potential of being utilized as markers, although further research is indicated to resolve the role of the cytokine network in knee OA diagnosis to reach concrete conclusions that can be generalized.

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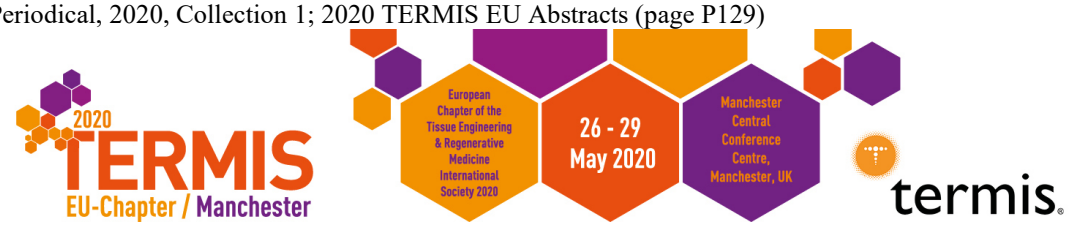
Keywords: Knee,



The Response of Primary Human Macrophages to Decellularized Cartilage Extracellular Matrix

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INTRODUCTION:The use of extracellular matrix (ECM)-based biomaterials in regenerative medicine has become common due to their cell-instructive properties and biodegradability. However, the host immune response, regulated by macrophages, can be a determining factor for successful regeneration after biomaterial transplantation. Macrophages are responsible not only for the inflammatory reaction by M1 but also for the process of constructive remodeling of the degradable biomaterials by M2 macrophages. In vitro assessment of macrophage response to a biological scaffold is a common way to estimate its suitability for further preclinical tests. Here we used an in vitro approach to evaluate polarization of primary human monocyte-derived macrophages (MDM) into M1 or M2 macrophages in response to the porcine nasal decellularized cartilage extracellular matrix (DECM).

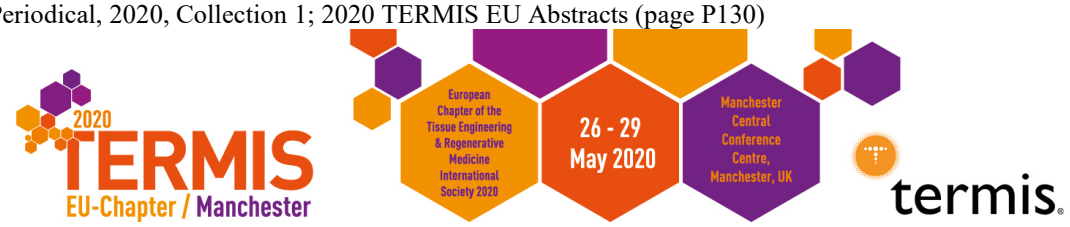
METHODS:MDM were cultured in the presence of DECM scaffold in a serum-free macrophage medium. As a reference, porcine ECM-based materials – small intestinal submucosa (SIS) and Parietex™ Composite (PRTX) were used. As a positive control, cells were treated with IFN γ or IL-4 to induce M1 or M2 phenotype, respectively. Conventional tissue culture polystyrene (TCPS) with no further treatment was used as a negative control. The MDM were harvested at culture days 1, 4, and 6 and analyzed for M1- and M2-specific surface marker expression using flow cytometry. Additionally, gene expression and multiplex analyses of inflammatory factors TNF-α, IL-1β, and CCL18 were performed. The culture was visually monitored by phase-contrast microscopy.

RESULTS:At culture day 1, MDM revealed a more spindle-shaped morphology in the presence of DECM compared to the other matrices. This distinction was, however, less visible at later time points. Higher TNF-α and IL-1β on both, gene expression and protein levels were observed in the DECM culture on day 1, as compared to the reference matrices. At later time points, however, the secretion of these factors was replaced by the production of the M2-specific factor, CCL18 in the DECM culture. The expression of the pro-inflammatory surface marker, CD38 was generally low in all conditions and was highest on day 1, particularly on TCPS and SIS. In contrast, the highest increase of the M2-specific marker, CD206 was seen with DECM as compared to the reference matrices.

DISCUSSION & CONCLUSIONS:Overall, while DECM induced MDM response, the initial inflammatory reaction did not persist and functional and phenotypical shift characteristic for M2 macrophages was observed.

Acknowledgements:Funding of the German Research Foundation DFG (Ro2207/5-1) is thankfully acknowledged.

Keywords: Decellularised matrices, Cartilage / joint and arthritic conditions



The Up-Scale Manufacture of Chondrocytes for Allogeneic Cartilage Therapies

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INTRODUCTION:Up-scaled allogeneic cellular products for cartilage therapies, along with identification of novel young donors of healthy chondrocytes, are needed to improve the cost effectiveness and wide-spread use of chondrocyte therapy.

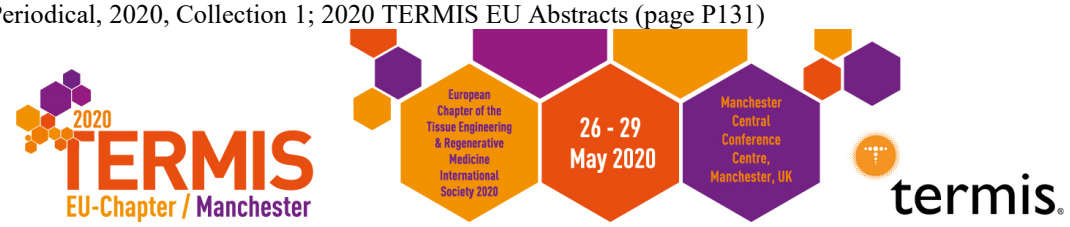
METHODS:Cartilage from four adult donors undergoing knee arthroplasty and two juvenile donors undergoing removal of extra digits were used to derive chondrocyte cultures in media supplemented with human platelet lysate (HPL, Stemulate®)) in the Quantum® hollow-fibre bioreactor or on tissue culture plastic (TCP) with foetal bovine serum (FBS) or HPL. Growth kinetics, flow cytometry and chondrogenesis assays were used to characterise cell products

RESULTS:The Quantum® produced higher yields of cells derived from arthroplasty patients (75±38M, in 9±1 days) cf. FBS supplemented (2.6±0.4M in 13.6±0.47 days) or HPL supplemented (6.9±3.8M in 11.0±2 days) TCP cultures. The number of population doublings for Quantum®, TCP FBS and TCP HPL was 2.82±1.18, 1.57±1.1 and 1.3±0.12, respectively. The same was seen in chondrocytes from juvenile donor digits (Quantum®: 74±37 M in 8 days; FBS supplemented TCP: 2.7±0.2M in 4±2.8 days; HPL supplemented TCP: 5.4±2.4M in 4±2.8 days). Quantum® adult donor chondrocytes displayed similar surface immunoprofiles to those grown on TCP and unlike freshly isolated cells, were immunopositive for CD90 (99%), CD73 (99%), CD105 (98%), CD166 (94%), CD151 (99%) and all integrins tested. Chondrogenic pellet analysis demonstrated that all adult donor cultures produced GAGs but to varying degrees.

DISCUSSION & CONCLUSIONS:The data presented here represents the first use of chondrocytes in a clinical-grade expansion bioreactor. We have demonstrated that compared to traditional cell expansion methods, the Quantum® is capable of generating higher numbers of chondrocytes. These Quantum® expanded adult chondrocytes demonstrate comparable characteristic immunoprofiles and cartilage forming capacity. The Quantum® has the potential to reduce manufacturing costs for multiple dose allogeneic chondrocyte banking. Further work will be performed to assess the potential of up-scale manufactured chondrocytes derived from juvenile digits.

ACKNOWLEDGEMENTS:Funding: Medical Research Council (MRC) Confidence in Concept Award (MC_PC_16049); MRC (MR/S015167/1) Versus Arthritis (19425; 20253; 21156).

Keywords: Cartilage / joint and arthritic conditions, Bioreactors



Differentiation of human articular chondrocytes in microtissues depends on the cell aggregation technique

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INTRODUCTION:Cell-based therapies were developed to treat joint cartilage defects since the self-repair capacity of this tissue is limited. However, chondrocytes lose their characteristic properties in vitro and need a 3D environment to support the production of a hyaline-like matrix. The success of this redifferentiation process varies depending on e.g. the donor and the method to generate microtissues. Hence, the aim of this study was to compare the chondrogenic maturation of chondrocyte-derived microtissues using different approaches.

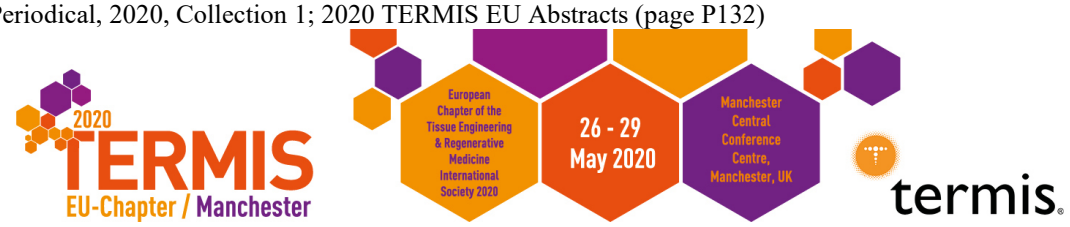
METHODS:Human articular chondrocytes isolated from knee joints were expanded in medium supplemented with human serum. Scaffold-free microtissues were generated using three distinct approaches: agar overlay technique, v-bottom plates (pellets) and cell-repellent v-bottom plates. The macroscopic appearance (reflected-light microscopy) was documented and the size was determined after two and four weeks cultivation. The differentiation degree was evaluated via immunohistochemistry (IH) to detect cartilage-specific markers (collagen type II, proteoglycans, Sox9) and collagen type I and via histology to visualise typical glycosaminoglycans (Safranin O, Alcian blue) on cryosections. The ATP level was quantified to determine the metabolic activity of chondrocytes in microtissues.

RESULTS:Microtissues were generated in all experimental setups; however, with distinct differences in the differentiation degree. A higher differentiation level was observed in microtissues cultured in both v-bottom plates compared to microtissues on agar. This was evidenced by a higher amount of extracellular matrix resulting in bigger microtissues (diameter on v-bottom plates was 80% larger compared to agar) and increased metabolic activity. Histological analysis revealed little/local staining on agar versus an overall staining on v-bottom plates with the highest intensity on cell-repellent plates. IH analysis of cartilage-specific markers showed the same pattern. The expression level of collagen type II and proteoglycans was enhanced in microtissues from v-bottom plates. However, Sox9 expression was distributed ubiquitously in all cultivation approaches. The dedifferentiation marker collagen type I was similarly expressed on a low level.

DISCUSSION & CONCLUSIONS:Our study revealed that the successful redifferentiation of chondrocytes in microtissues is directly linked to the method for the induction and development of spheroids. The chondrogenic profile of microtissues in cell-repellent v-bottom plates is superior to the other techniques. Therefore, v-repellent plates are recommended for the generation of microtissues with a distinct chondrogenic phenotype and avoiding foreign substances (agar) or physical stress (centrifugation). These microtissues seem to be quite suitable as transplants to regenerate cartilage defects.

ACKNOWLEDGEMENTS:This work had been supported by „Gesundheitscampus Brandenburg“ and „Ministerium für Wissenschaft, Forschung und Kultur“, state of Brandenburg, Germany.

Keywords: Cell therapy, Differentiation



A preliminary study testing fibrin glue as cell delivery vehicle for synthetic meniscus tissue engineering

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INTRODUCTION:Fibrin glue facilitates cell attachment and tissue formation when applied surgically for a number of different applications including cartilage repair cell-based therapies. This study was designed to test the feasibility of using fibrin gel as cell delivery vehicle to seed chondrocytes into the commercial meniscus scaffolds Actifit® (polyurethane and PCL) and CMI® (collagen meniscus implant), both of which are used acellularly in the clinic for meniscus replacement strategies, but with limited success.

METHODS:Actifit and CMI scaffolds were sectioned into 4mm width pieces. Chondrocytes harvested from a total knee replacement cartilage donor (P5, 1 million/scaffold) were seeded into Actifit and CMI with or without fibrin gel (fibrinogen 20mg/ml: thrombin 100U/ml= 4:1). The scaffolds were cultured in media for two weeks. Cytotoxicity assays were performed at day1, day7 and day14 to assess the cell proliferation rate. Histology and confocal microscopy were used to determine cell distribution and viability.

RESULTS:Light microscope imaging confirmed that the cell retention within the scaffolds was enhanced by fibrin gel in both groups compared to scaffolds without fibrin gel. This result was corroborated by cytotoxicity assays. The histological analyses showed that a more even cell distribution was observed with fibrin gel in the CMI group compared with the Actifit group. The confocal analysis suggested that more dead cells were found in the CMI group compared to the Actifit group without fibrin gel, while better cell viability were observed in both scaffolds with the inclusion of fibrin gel.

DISCUSSION & CONCLUSIONS:This preliminary study suggests that fibrin glue may better sustain the survival of implanted chondrocytes in commercial synthetic meniscus scaffolds in vitro. In future work, we will test the performance of cell – fibrin gel – scaffold mixtures under perfusion and cyclic compression using a bioreactor to mimic in vivo conditions, compared to static cultures.

ACKNOWLEDGEMENTS:Versus Arthritis, the CRMI Technology Centre (Oxford), China Scholarship Council and the Institute of Orthopaedics for funding. Joint Operations for the provision of scaffolds for this research.

Keywords: Cartilage / joint and arthritic conditions, Cell therapy

Effect of a novel, arthroscopic cutting device and commercial surgical tools on the viability of articular cartilage

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INTRODUCTION:Mechanical injury of articular cartilage, causing tissue damage and chondrocyte death, can occur during debridement, drilling or cutting the cartilage during operative procedures. Impaired integration of repair tissues with native cartilage may occur if chondrocyte death is observed in the integration zone[1]. We are developing a novel cutting-device for arthroscopic use in confined joints (e.g. hip). This tool is designed to remove defective tissue from chondral or osteochondral lesions while causing minimal damage to the surrounding cartilage prior to insertion of a regenerative medicine or cell therapy product. The aim of this study was to establish an in vitro model to assess the effect of the prototype-cutting heads and commercial orthopaedic surgical tools on the quality of the cut and cartilage viability

METHODS:Bovine metacarpophalangeal joints were dissected and the M3/M4 bone removed. A plug of chondral or osteochondral tissue was removed from the two distal ridges of a knuckle with the selected cutting tool. The knuckle end was removed and transferred to protein-free DMEM (2h, 37oC) to enable an injury response to develop. The knuckle was then incubated (1h, RT) in protein-free DMEM containing 5µM Cell Tracker Green™ (to stain live cells) and 5µM propidium iodide (to stain dead cells). The knuckles were washed with PBS, fixed in buffered formalin and confocal microscopy used to detect live and dead chondrocytes

RESULTS:Scalpel cuts showed occasional dead chondrocytes along the cut tissue, similarly. Compared to our early designs (clean-edged cuts, some chondrocyte death), the final prototype cutting tool effectively removed chondral and osteochondral tissue to leave a clean-edged hole microscopically and macroscopically and without a zone of dead chondrocytes. The commercial surgical tools gave smooth, clean-cut profiles holes macroscopically which were ragged-edged microscopically. The 4mm orthopaedic open curette gave 50-200µm zone of dead chondrocytes around the hole; a 13mm orthopaedic closed curette gave a zone of cell death of 200µm; a 6mm OATs reamer tool (recommended to prepare the surgical site for receiving a cartilage graft) a surrounding zone of tissue damage up to 500µm width; and a 5.5mm shaver gave a ragged profile cut macroscopically and microscopically, with little dead tissue around the cut edges.

DISCUSSION & CONCLUSIONS:This model enabled assessment and development of design iterations of our cutting tool to yield a design which maximised cartilage viability around the cut above that of the commercial tools tested in this system.

ACKNOWLEDGEMENTS:Innovate UK for funding this study

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Keywords: Trauma / surgery and rehabilitation, Wound healing

Biological, Structural and Functional Properties of Human and Elastic Cartilage Microtissues

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INTRODUCTION:Dedifferentiation during cell culture is one of the main factors related to the loss of cell functionality and failure of cell-based therapies in cartilage tissue engineering (1). Generation of 3D microtissues (MT) by using non-adherent molds, such as agarose, is a promising alternative to favor cell function and differentiation, promoting extracellular matrix synthesis and decreasing the degree of cell dedifferentiation (2). In this study, we generated and analyzed the biological, structural and functional properties of human elastic and hyaline cartilage MT.

METHODS:Elastic and hyaline chondrocytes were isolated from human biopsies of both types of cartilage. MT were generated by seeding chondrocytes in agarose moulds containing 1250 micro-wells with 400 µm of diameter each. Cells were kept under conventional culture conditions with expansion medium and the histological pattern, immunohistochemical markers and cell metabolic activity were analysed after 4, 7, 14, 21, 28 days of ex vivo development.

RESULTS:With both cell types, we were able to generate stable MT from day 7 onward. The morphology, dimensions and circularity over the time were more stable and constant with hyaline chondrocytes than with elastic cells. Analysis of cell viability with Live/Dead and WST-1 confirmed an acceptable degree of cell viability and functionality in both experimental conditions, especially with hyaline chondrocytes. Similarly, histology confirmed consistent and well-organized MT with the use of hyaline chondrocytes, which were more stable than elastic chondrocytes, which tended to dissociation over the time. A consistent positivity for S-100 was observed in both cells types, but collagen type II was more abundant when elastic chondrocytes were used.

DISCUSSION & CONCLUSIONS:This demonstrated that both cell types are suitable for the generation of 3D MT for cartilage tissue engineering, being more stable and compact with the use of hyaline chondrocytes. In addition, this study demonstrate that MT were viable and positive for S-100 during the 28 days analyzed. However, the slight reaction for collagen type II could indicate certain degree of dedifferentiation over the time, especially with the use of hyaline chondrocytes. Future studies are needed to demonstrate the usefulness of these MT in the generation of biomimetic and functional substitutes for elastic and hyaline cartilage repair.

ACKNOWLEDGEMENTS:This study was supported by Grant CS PI-0257-2017 from Consejería de Salud y Familias, Junta de Andalucia, Spain.

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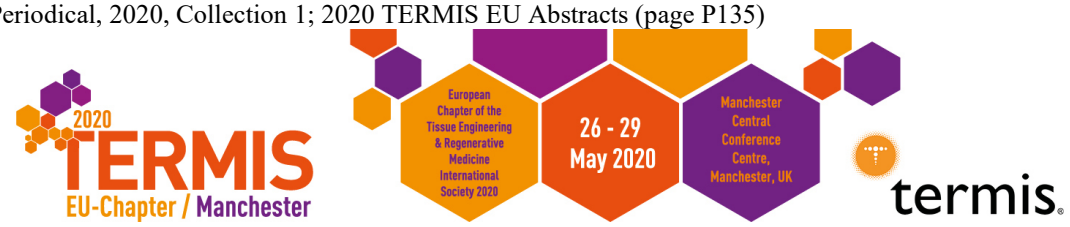
Keywords: Cartilage / joint and arthritic conditions, Biofabrication



Regenerative therapy of bronchial fistula

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INTRODUCTION:Bronchial fistula is still the most serious complication following lung resection and associated with serious management problems, and high rates of mortality. The therapeutic approach is individualised and varies with the type of fistula. The standard treatment is based on surgical closure or endoscopic techniques and inefficient. We attempted to develop a new treatment for bronchial fistula using regenerative therapy including autologous platelet lysate and allogeneic dermal fibroblasts.

METHODS:This study included 34 patients with bronchial fistula after lung surgery. Patient characteristics such as age, sex, fistula type and previous operations were recorded. The local ethical committee approved this study and written informed consent was obtained from all patients. After the detection of clinical and bronchoscopic signs of bronchial fistula patients were included in the clinical trial. The regenerative product, consisting of human allogenic dermal fibroblasts suspension and autologous platelet lysate, was injected into the cavity of the fistula through a bronchofibroscope every second day totally five times. Hystological examination of cells from biopsies was performed after each bronchoscopy. Human allogeneic dermal fibroblast were obtained from GMP laboratory. The fibroblasts were isolated and grown following the standard operating procedures and storage under liquid nitrogen condition at -196C before use. The total number of cells used in the one injection was 2 million per ml. Before the bronchoscopy 50 mL of whole blood was collected. Platelet rich plasma was obtained by two-step centrifugation the patient's blood and collecting platelet concentrate suspended with plasma of total volume 10 ml. Freeze/thaw cycles were used for activation platelet. The aliquots of 2 ml platelet lysate were stored at −80oC until use.

RESULTS:Thirty four patients, of whom 33 (97%) were male, were treated according to protocol. Median age was 59±9,6 years, and ranging from 22 to 71. The bronchial fistula was localized in main bronchus in 23 (67%) cases, in peripheral bronchus – 8 cases. From 34 patients 29 had the diameter of fistula less 10 mm, and in 5 cases the full failure of bronchial stump was determined. All patients underwent bronchoscopy after treatment. All fistulas closed within 1 months after beginning of regenerative therapy. Bronchoscopic image confirmed fistula healing. No recurrence developed after definitive treatment. There were no adverse events. The histological examination revealed the formation of granulation tissue, metaplastic squamous epithelium.

DISCUSSION & CONCLUSIONS:Based on the results of this study, a regenerative therapy of bronchial fistula appears to be a promising treatment modality.

Keywords: Trauma / surgery and rehabilitation, Personalised medicine

Enhanced Bone Marrow derived mesenchymal Stem cell differentiation when isolated and expanded with human Platelet Rich Plasma and differentiation media is supplemented with Vitamin D

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INTRODUCTION:Vitamin D3 is well known to be involved in bone formation during foetal development and has been shown to be actively involved from gestational day 13 during foetal rat development [1]. Vitamin D3 and its associated enzyme 1-alpha-hydroxylase a member of the cytochrome P450 super family and encoded for by the gene CYP27B1. In this study we supplemented osteogenic media with vitamin D at a concentration of 10-4M and differentiated for 21 days with samples analysed by means of quantitative alizarin red assay and qPCR for the bone markers RUNX2, ALPL and HPRT1

METHODS:Lonza bone marrow aspirate was seeded at 104 cells per CM3 and isolated and expanded in either FBS or human platelet rich plasma with high glucose glutamax™ DMEM supplemented with 1% non-essential amino acids, 1% penicillin, streptomycin and amphotericin C. once isolated hMSCs were characterised using hMSC antibody panel (R&D systems). hMSCs were then seeded for tri-lineage differentiation with time points of 0, 3,6,9,12,18, and 21 days.Osteogenic media was further supplemented with vitamin D at a concentration of 10-4M. Osteogenic differentiation was quantified using quantitative alizarin staining along with qPCR.

RESULTS:Preliminary results have shown no significant difference was observed over the first 18 days between any of the samples. On comparing FBS directly with hPRP isolated and expanded then differentiated mesenchymal stem cells. Basal differentiation media and osteogenic differentiation media showed no significant differences between FBS versus hPRP over the 21 days. Conversely, on comparing FBS and hPRP when differentiated with osteogenic media supplemented with vitamin D, hPRP had a significantly higher calcification of 2.75 ± 0.276 ($p=0.0032$) compared to FBS calcification of 0.043 ± 0.013 for day 18. For day 21 hPRP calcification had a value of 2.75 ± 0.275 which was significantly higher ($p=0.0003$) when compared to the FBS values of 0.34 ± 0.08. qPCR results are still being evaluated with early results showing hPRP isolated cells showing up regulation of RUNX2 and associated bone markers by day 9, conversely FBS cultured expanded cell still show lower expression of ALPL and RUNX2.

DISCUSSION & CONCLUSIONS:From our early results we have shown clearly that calcification occurs earlier with hPRP expanded cells when differentiated in the presence of Vitamin D, which is also observed when hMSCs are isolated and expanded in FBS. Our early data indicates that vitamin D may be vital for bone differentiation.

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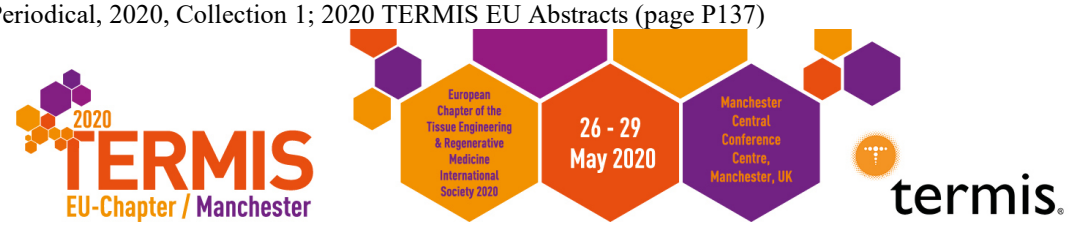
Keywords: Cell therapy, Developmental biology



Development of quality discrimination method for Human adipose-derived stem cells based on cell morphology information

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INTRODUCTION:Human Adipose-derived Stem Cells (hADSCs) can be collected by means such as subcutaneous liposuction, and thus is less invasive. Furthermore, high healing ability has been reported for liver diseases and damaged skin regeneration, and its application as a pharmaceutical is being promoted [1, 2]. However, a quality evaluation method in cell production has not been established, and a highly efficient and stable evaluation system is required.

METHODS:The growth and differentiation potential of hADSCs with different passage numbers were evaluated experimentally, and cellular images over 12000 were acquired for extracting morphological changes due to passage numbers. For the quality evaluation models, the prediction of “potency lacking status”, the performances were visualized by principal component analysis (PCA) and Mahalanobis-Taguchi (MT) method.

RESULTS:From PCA analysis, it was clear that the multiple morphological parameter profile changed in correlation to the decrease of their potencies, and out both visualization scheme could sensitively detect the irregular quality decay in hADSCs.

DISCUSSION & CONCLUSIONS:Our results show that the qualities of hADSCs in the manufacturing process can be modeled with good accuracy by comprehensive imaging and information analysis, therefore can be applied to the daily quality monitoring for sensitive label-free detector for cellular product manufacturing.

Acknowledgements:Authors are grateful for financial support from the Next Generation AI Technology Field: Strategic Advancement of Multi-Purpose Ultra-Human and AI Technologies (SamuRAI) from New Energy and Industrial Technology Development Organization (NEDO).

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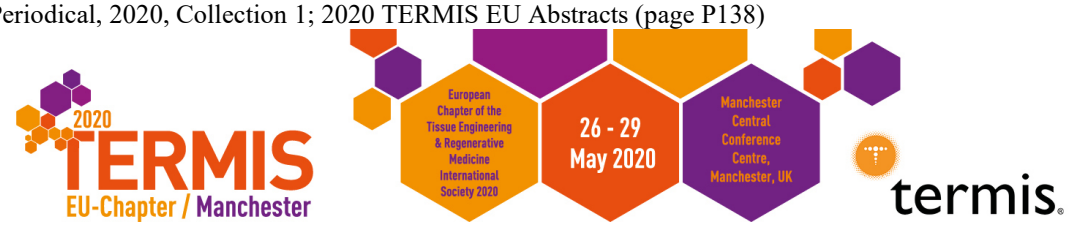
Keywords: Multipotent (mesenchymal) stem cells, Imaging – advanced



Data analysis for stereotype morphology for robust evaluation of MSCs

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INTRODUCTION:In recent year, human cells are new types of medicinal products that are rapidly growing. Human mesenchymal stem cells (MSCs) had started to be approved as both autologous and allogenic cell therapeutic products in the world. Human adipose derived MSCs (hADSCs) has an advantage of feasible access to the origin tissue, and alto has the multi-potent for differentiation and immunomodulatory effects [1]. Some reports show that ADSC can provide higher yield in their manufacturing [2]. By such scientific developments, there are growing demands for evaluating the quality of culturing cells non-invasively by images for better quality control in cell manufacturing. Our group has been reporting the morphology-based cell quality evaluation using microscopic imaging system and machine learning. However, in the usage of machine learning in such morphological images, the biggest difficulty was the limitation of trained computer for new data to face the large difference in individual differences. We here report a new morphological data machine learning concept to reduce the experimentally volume but enhance the constructed models' performance.

METHODS:Using hADSC lot library with variations of passages, we collected the hADSCs' morphological database from more than 10000 cells covering more than 20000 images, and processed the data to extract the commonly shared "stereotype morphology" by data analysis. By using these stereotyped data of morphology, we tried to construct morphology-based prediction models to predict their decrease in quality.

RESULTS:Our morphological data analysis revealed some preference rules that is especially found in early/late passage samples, and such extracted morphologies enhanced the prediction performance.

DISCUSSION & CONCLUSIONS:Our data showed to use the most of the limited data, by introducing data analysis in multi-parametric morphological parameters for robust machine learning, and it can be applied to the cell manufacturing processes to predict their cellular quality from limited cells.

Acknowledgements:Authors are grateful for financial support from Research Study on Next Generation AI Technology Field: Strategic Advancement of Multi-Purpose Ultra-Human and AI Technologies (SamuRAI) from New Energy and Industrial Technology Development Organization (NEDO).

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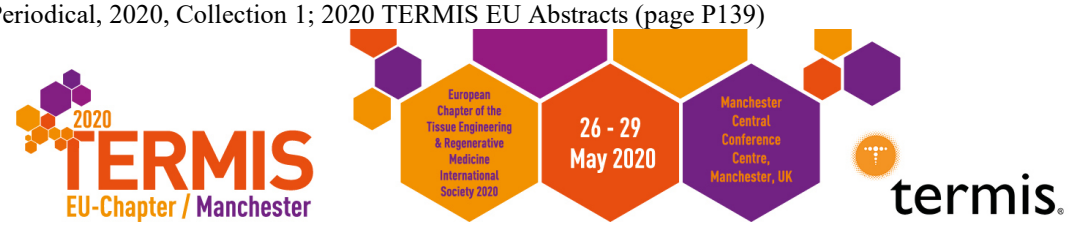
Keywords: Multipotent (mesenchymal) stem cells, Imaging – advanced



Cellular viroimmunotherapy migrates to the tumor site in a canine patient

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INTRODUCTION:The use of oncolytic virotherapy as new approach for cancer has shown promising results. However the intravenously (i.v.) administration of the agent in some occasions presents a challenge. In order to improve the i.v. use of oncolytic adenovirus (Ads), our group developed the use of mesenchymal stem cells (MSCs), infected with Ads, as “Trojan horses” to be administrated i.v. as an antitumoral immunotherapy (called Celyvir). We reported the efficacy of this strategy in mice and canine and human patients. In a veterinary clinical trial, 27 canine patients were treated with canine Celyvir (dCelyvir) showing a response rate of 74%, including 14.8% of complete remissions. The homing capacity of infected-MSC has been described in murine models but never in patients. Here, we perform an in vivo biodistribution study of dCelyvir in a canine patient with a spontaneous lung carcinoma. This is the first time that it is reported that marked/infected-MSCs are detected inside of the tumor 48 hours after i.v. inoculation.

METHODS:To carry out the biodistribution study, dMSCs were labeled with GFP and infected with a canine oncolytic adenovirus. Then, patient was treated with dCelyvir-GFP and 48 hours after the i.v. administration, tumor was removed by surgery and we analyze the presence of MSC and adenovirus. Tumor was analyzed to detect GFP fluorescence by microscopy and flow cytometry and we also stablish a cellular culture. Further, in the 1st, 3rd, 7th and 14th day after the dCelyvir-GFP treatment, peripheral blood samples were taken to detect and quantify the oncolytic adenovirus by qRT-PCR.

RESULTS:Green fluorescence cells were detected on the cellular culture by microscopy. Further, 0.69% of the cells from the tumor sample were GFP-positive analyzed by flow cytometry. The presence of the adenoviral DNA on peripheral blood was confirmed by qRT-PCR the first day after treatment and the DNA amount progressively goes down.

DISCUSSION & CONCLUSIONS:For the first time Ads infected-MSC has been observed inside of the tumor after i.v. inoculation in an oncologic patient, confirming the tumoral homing capacity of infected-MSC in a clinical setting.

Acknowledgements:We thank the Hospital Veterinario Madrid Norte that kindly provided samples of adipose tissue to obtain MSCs.

Keywords: Cell therapy, Cancer

Therapeutic efficacy of M2 macrophages for aortic aneurysm

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INTRODUCTION:We previously reported that intravenous injection of MSCs inhibited expansion of aortic aneurysms (AA) due to improvement of imbalance of M1 / M2 macrophages which might be induced by MSCs paracrine effects. Focusing on anti-inflammatory properties of M2 macrophages (M2MF), we examined whether an already-formed AA can be treated by M2MF in a mouse model.

METHODS:AA was induced in apolipoprotein E-deficient mice by angiotensin II-infusion for 28 days through subcutaneous osmotic mini-pumps. After that, 10^6 M2MF (in 0.2 ml saline) or 0.2 ml saline as a control was injected intraperitoneally. Mice were sacrificed at 4 weeks (saline group n = 10, M2MF group n = 10) after injection. The AA tissues of each group were dissected and assessed for aortic diameter, elastin area, endogenous active matrix metalloproteinase (MMP)-2 and -9, macrophage infiltration, and PKH-26 labeled M2MF tracking.

RESULTS:Compared with the saline group, the M2MF group reduced aortic diameters (1.5 vs 2 mm, $P < 0.001$), endogenous active MMP-9 (4 vs 2.7 ng/mL, $P < 0.05$) and inflammatory cytokines including IL-1β (71 vs 220 pg/ml, $P < 0.05$), IL-6 (88 vs 142 pg/ml, $P < 0.05$), TNF-α (13 vs 27, $p<0.05$), MCP-1(76 vs 111 pg/mL, $p<0.05$), and ratio of M1/M2 (1 vs 2.2, $p<0.05$). In addition, the M2MF group up-regulated elastin area (55 vs 46 %, $p<0.05$) and the expression of cytokines including IL-4 (65 vs 34 pg/ml, $p<0.05$) and IL-10 (30 vs 17 pg/mL, $p<0.001$). Injected PKH-26 labeled M2MF was observed in the AA wall and maintained expression of CD206 antigen.

DISCUSSION & CONCLUSIONS:Our results suggested that M2MF inhibited AA expansion and might be an effective as a new cell therapy tool.

Keywords: Cardiovascular, Cell therapy

Comparative analysis of human limbal cells cultivated on different type of feeder layers for cell therapy

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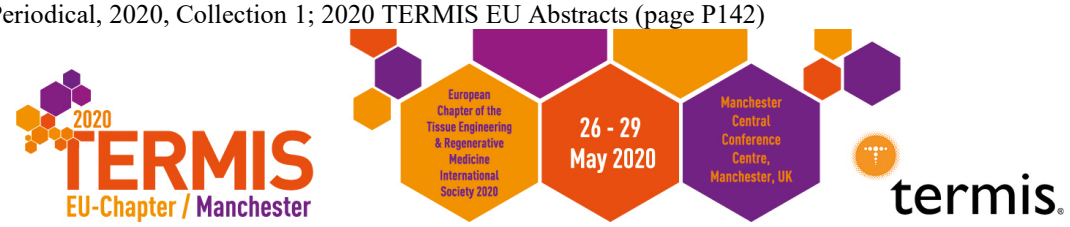
INTRODUCTION:Cultivation of limbal stem cells (LSC) is a well-established method of cellular therapy, classified as advanced therapy medicinal products (ATMPs), for patients with limbal epithelial stem cell deficiency (LSCD). Our aim was to compare human LSC cultivated with growth-arrested mouse 3T3 fibroblasts as feeder layer (FL) cells prepared in two different ways, treated with mitomycin C and gamma irradiation. All procedures were followed by good manufacturing practice and quality control.

METHODS:We describe the validation results of human LSC grown in vitro in respect of growth, viability, cell morphology, colony forming efficacy (CFE) and holoclon forming efficacy (HFE). For the CFE and HFE assays human limbal epithelial cells were seeded at different densities (1000, 1500 and 2000) co-cultured with growth-arrested FL cells in 100 mm Petri dishes. Colonies were fixed and stained on the 13th day at room temperature with crystal violet. Cell growth and morphology of LSC on human amniotic membrane was monitored with a phase-contrast microscope. These limbal grafts were additionally evaluated by histopathological and immunohistochemical analysis after eight days of cultivation. Assessment of limbal graft contamination with mouse FL cells was acquired by a flow cytometer using anti-FL monoclonal antibody whereas bacterial endotoxin presence was evaluated using limulus amebocyte lysate (LAL) assay.

RESULTS:Under cell culture conditions the proliferation of limbal cells was followed in different types of a FLs (with mitomycin C or gamma irradiation) and exhibited similar morphology, viability (95%) and CFE (2.7-3.4%). Immunohistochemical analysis detected positive staining of stem cells (p63) and a marker of stem cell proliferation (Ki67) while epithelial differentiation was evaluated using citokeratyn 3 expression (CK3) on the limbal graft. Histological analysis demonstrated the ability of generating stratified epithelium. LSC cultivated on FL prepared with mitomycin C had greater HFE (0.3-0.4%) and higher cell density on the limbal graft compared to LSC cultivated on FL prepared with gamma irradiation (0.2-0.25%). Content of FL cells in limbal grafts were below < 5% and concentration of endotoxin was < 0.250 EU/mL.

DISCUSSION & CONCLUSIONS:Limbal cells cultivated on FL cells inactivated by mitomycin C or gamma irradiation have produced similar results in regard to cell morphology, viability, proliferative capacity, CFE, endotoxin concentration, stratification and expression pattern of p63, Ki67 and CK3. However, LSC co-cultured with FL prepared with mitomycin C were superior in promoting holoclon formation potential and cell density on the limbal graft.

Keywords: Cell therapy, Eye



Mesoangioblast-Endothelium interactions regulate engraftment in cell transplantation protocols for muscular dystrophy

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INTRODUCTION:Many new therapies are being tested for MD but none has yet reached clinical efficacy. Our lab pioneered stem cell transplantation that led to a first in man trial in DMD patients. The first trial was safe but not efficacious [1]. This project aims to implement critical steps of mesoangioblasts (MABs) transplantation. We want to study and positively modulate their diapedesis and extravasation.

METHODS:An in vitro IBIDI microfluidic assay, mimicking blood stream dynamic, allows the track in real time MABs adhesion to the endothelium. Through this system TNF inflammatory activity, and deflazacort (DFZ) anti-inflammatory effect at the single cell level have been confirmed.

RESULTS:When activated via TNFα, HUVECs over-express VCAM-1, I-CAM, and E-Selectin adhesion molecules at both mRNA and protein level. Expression was inhibited by the anti-inflammatory effect of DFZ. MABs-adhesion ability increases about 80 % after pre-activation of HUVECs with TNFα for 6 hours. By pre-treating the endothelium with both TNFα and DFZ, adhesion events were reduced of about 40 % and 80 % after respectively 6 or 24 of hours treatment. Therefore, MABs ability to interact with HUVECs is specifically dependent on the endothelium status, resulting in a decrease of MABs attachments in the presence of steroids.

DISCUSSION & CONCLUSIONS:These results will be confirmed in vivo, through experiments conducted on dystrophic mice under steroid administration. Perivascular ECM reconstruction will elucidate the migration mechanism adopted by these cells to reach the dystrophic muscles. The results may have important implications for future protocols based upon intra-vascular cell delivery for DMD.

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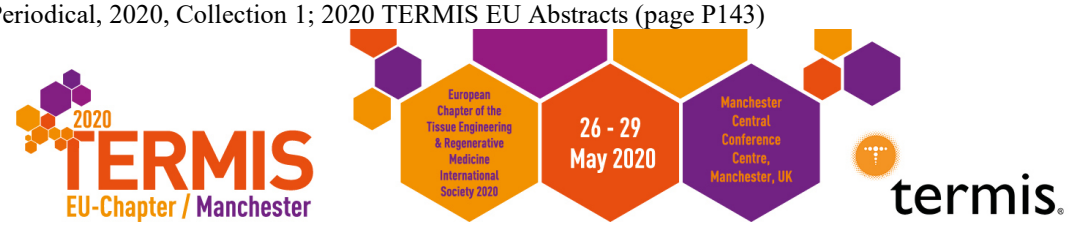
Keywords: Cell therapy, Musculoskeletal (inc ligament / tendon / muscle / etc)



Bioceramic cylinders as a scaffold in ectopic bone formation induced by rhBMP6 in autologous blood coagulum

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INTRODUCTION:In our previous work we have shown that autologous blood coagulum (ABC) is a novel carrier for bone morphogenetic protein 6 (rhBMP6) which is an important molecule in bone regeneration because it posseses osteoinductive properties. Compression resistant matrix (CRM) is used to improve biomechanical properties of the novel autologous bone graft substitute (ABGS). The aim of this work was to elucidate time course of ectopic bone formation following subcutaneous implantation of osteoinductive device containing recombinant human BMP6, ABC and biphasic bioceramic cylinders (80% tricalcium-phosphate/20% hydroxyapatite) as CRM.

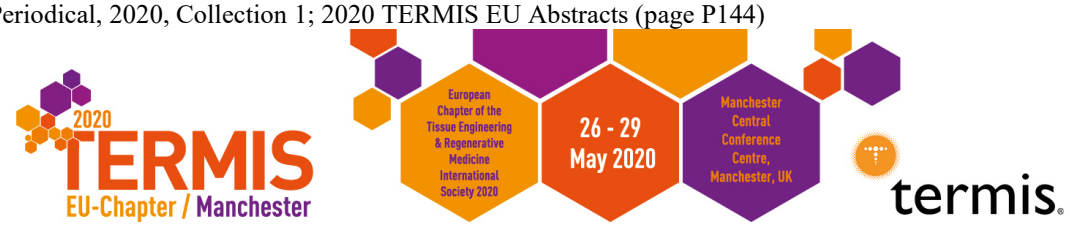
METHODS:In the process of preparation of the ABGS with osteoinductive properties, 20µg of rhBMP6 was added to 500µL of ABC and coagulated in a syringe containing cylinder (0,1g). Implants were implanted subcutaneously (n=6 per time point) in the axillary region of Sprague Dawley rats and removed on specified time points (7,14,21,35 and 50 days). Histological examination was used to assess the biological events in ectopic bone formation and remodeling. The new ectopic bone was analysed by µCT.

RESULTS:On day 7 after implantation endochondral ossification was present outside the cylinder in the peripheral part of implants. On day 14 and day 21 following implantation newly formed bone was present both around the cylinder and in the majority of pores inside the cylinder. Bone marrow was present between the bone trabeculae. Histological findings on day 35 and day 50 were almost similar. Newly formed bone almost completely surrounded the cylinder and was also present in the pores. Bone was uniformly present in the pores regardless of the pore location inside the cylinder and covered the surface of the bioceramics encircling pores with bone. Within the osseus circle, there were few trabeculae and bone marrow with predominance of adipocytes. Ectopic bone formation was confirmed by µCT analyses.

DISCUSSION & CONCLUSIONS:In this experiment we investigated time course of ectopic bone formation induced by rhBMP6 which is a part of the aforementioned ABGS. Sequence of biological events as well as amount of newly formed bone were comparable with the findings of our previous experiments in which various bioceramic granules were used as CRM. We have shown that bioceramic cylinders might be used as a CRM in osteoinductive ABGS containing rhBMP6, ABC and CRM. To comfirm the possible use of the tested ABGS, it will be tested in larger animals.

ACKNOWLEDGEMENTS:This work was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine (Grant Agreement KK01.1.1.01.0008)

Keywords: Biologics and growth factors, Biomaterials



Tailoring the bioactivity of an extracellular matrix-based material by engineering composite materials of cell-derived extracellular matrix and macromolecules

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INTRODUCTION:Application of tissue-derived extracellular matrix (ECM) has risen as one of the most successful therapies for the treatment of chronically inflamed tissues, such as chronic wounds. However, much improvement is still needed as these ECMs possess fixed bioactivity and their incomplete decellularization poses a risk of rejection and disease transmission. To overcome these limitations we aimed to construct a composite material based on cell-derived ECM and the heparan sulfate-mimetic dextran sulfate (DxS-ECM). We hypothesize that the combination of the anti-inflammatory properties of mesenchymal stromal cells' (MSCs) secretome and DxS's bioactivity will result in an ECM-based material with enhanced anti-inflammatory properties.

METHODS:The interaction between DxS (500 kDa) and major ECM components fibronectin and collagen was investigated by dynamic light scattering (DLS) and the effect of DxS on ECM deposition was examined on day 2 and 6 in MSCs cultures. ECM-rich cultures were decellularized resulting in a composite material (dDxS-ECM) composed of ECM and DxS. The material was further investigated for its anti-inflammatory properties in macrophage cultures.

RESULTS:DLS measurements of mixtures of DxS and ECM components revealed that DxS forms aggregates with fibronectin and collagen I. This could be further confirmed by immunostaining of cell cultures, where co-deposited DxS-ECM aggregates were visualized in the cell layer. In accordance, MSC cultures supplemented with DxS were rich in collagen I and fibronectin, leading to a significantly higher production of ECM in comparison to their un-supplemented counterparts.
Next, the composite material was investigated for its anti-inflammatory properties in an in vitro assay with THP-1-derived macrophages under pro-inflammatory stimuli. Macrophages interacting with dDxS-ECM secreted lower levels of pro-inflammatory cytokines when compared to control materials.

DISCUSSION & CONCLUSIONS:DxS was able to co-aggregate and deposit with ECM components and consequently enhance ECM deposition. The here presented dDxS-ECM composite material was tailored to downregulate the inflammatory response in the presence of inflammatory stimuli and is therefore a promising cell-derived ECM-based material for application in ischemic and pathologically inflamed microenvironments, such as in chronic wounds.

Keywords: Decellularised matrices, Immunity / immunomodulation / macrophage

Biomimetic Mineralization of Silk Fibroin Scaffolds for Bone Tissue Engineering

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INTRODUCTION:Bone has remarkable mechanical properties thanks to its organized mineralized collagen matrix. Bone tissue engineering aims at mimicking this structure for the development of in vitro bone models or implantable grafts, making use of biomaterials like silk fibroin scaffolds. However, silk fibroin only mimics the organic extracellular matrix component of bone. These scaffolds lack the bone-like mechanical characteristics that are important for osteogenesis and osteoclastogenesis. In vivo, non-collagenous proteins play an important role in the infiltration of mineral into the collagen [1]. In vitro, poly-aspartic acid (pAsp) is often used to mimic the functionality of non-collagenous proteins [2]. Here, we use pAsp to enhance the mineralization of silk fibroin scaffolds and to improve infiltration of minerals into the silk fibroin, with the aim to better mimic the in vivo bone structure and function for bone tissue engineering applications.

METHODS:Pure silk fibroin scaffolds and silk fibroin scaffolds containing 5 wt% pAsp were mineralized using a solution of 10x simulated body fluid (SBF) [3] or 10x SBF with 50 ng/ml pAsp. After 7 days, scaffolds were harvested and characterized with µCT and SEM. Calcium content was quantified biochemically and localized with alizarin red staining. To quantify mineral precipitation in the mineralization solution, its optical density was measured.

RESULTS:Mineral precipitation in solution was reduced in the groups with pAsp in the mineralization solution. Mineralization of the scaffolds under these conditions was enhanced and the mineral appeared also in the scaffold structure. When pAsp was only present in the scaffold the mineral only precipitated on the surface of the scaffolds. The combination of pAsp in the scaffold and solution led to enhanced mineralization, with minerals both on the surface of the scaffold and within the scaffold structure.

DISCUSSION & CONCLUSIONS:Addition of pAsp to the mineralization solution and integrated into the scaffold can enhance the mineralization of silk fibroin scaffolds and improve mineral infiltration. The next step will be to investigate whether this mineralization method leads to improved osteogenesis and osteoclastogenesis, and subsequent extracellular matrix formation and resorption.

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Keywords: Biomaterials, Bone and bone disorders (osteoporosis etc)

"Control of supramolecular helicity by the energy input and the monomer stereochemistry"

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INTRODUCTION:Glycosylation of proteins is a common post-translational modification by which glycans of different complexity are grafted onto the protein core. Glycosylation is involved in the regulation of different cellular processes, such as adhesion, differentiation and proliferation, or activation of pathologic pathways. In fact, a modified glycosylation patterns (e.g. truncation) have been associated with neurodegeneration, inflammation, auto-immune activity and cancer.1-2

METHODS:To generate the monomer units, we used several monosaccharides, namely glucosamine (GlcN), galactosamine (GalN) and mannosamine (ManN) that differ by their stereochemistry and modify them with aromatic moiety (Fmoc) to impart amphiphilic properties.3 We studied the assembly of FmocX (X = GlcN, GalN or ManN) upon different energy inputs. While live systems use ATP as energy input, we used temperature due to possibility of tighter and simple control.
The amphiphiles were dissolved in hot (90 ºC) water and their supramolecular assembly was studied by AFM, SEM, CD and fluorescence spectroscopy upon cooling to room temperature at different rates (i.e. fast, 40 ºC/min or slow, 5 ºC/min).

RESULTS:FmocGlcN and FmocGalN formed nanofibrous hydrogels upon cooling, regardless of the energy input (i.e. fast and slow cooling regime). In contrast, FmocManN was not soluble in aqueous media. The sol-gel transition occurs above the physiological temperature between 38-51°C and Tgelslow cooling >Tgelfast cooling. CD spectra showed a positive signal generated from either FmocGalN or FmocGlcN at fast cooling. Interestingly, at slow cooling, there was an inversion of the CD signal only for FmocGalN. The nucleation and growth of the nanofibers were also influenced by the stereochemistry: FmocGalN form less but longer fibres while more but shorter fibres were observed for FmocGlcN. These differences affected the viscoelastic properties of the generated gels: FmocGlcN have higher storage modulus than their FmocGalN counterparts. The storage modulus (G') was also affected by the energy input: G'slow cooling>G'fast cooling.
DISCUSSION & CONCLUSIONS:We developed a reductionist model of glycoproteins by which we show that the carbohydrate stereochemistry and energy input influence the assembled architectures. The obtained results recapitulate important nano- and macro-scaled features related with the glycosylation of the proteins and the related bioactivity.
Acknowledgements:We acknowledge the financial support from the EC (#668983-FORECAST, #739572-THE DISCOVERIES CTR and #872648-MEPHOS) and FCT (PhD grant PD/BD/135256/2017, INCIPIT M-ERA-NET2/0001/2016).
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Keywords: Biomaterials,

Humidity-controlled folding of poly(e-caprolactone) agarose hydrogels composites

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INTRODUCTION:Materials that can be actuated by external stimuli are critical for the manufacturing of interactive objects. These materials open the possibility to change the shape of objects in response to external conditions, such as humidity. Biocompatible and biodegradable materials like agarose and poly(ε-caprolactone) (PCL) are of high interest as they can lead to objects able to interact with biological systems for instance for the implantation of small print-size objects that can deploy under physiological conditions. In this study, we describe the design of agarose composites hydrogels that upon dehydration automatically fold into a predictable shape guided by a PCL scaffold embedded within the hydrogel.

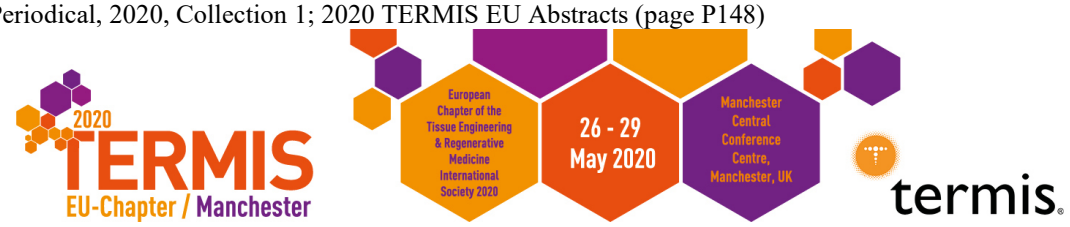
METHODS:Scaffolds with notch-inducing deformations were 3D printed in PCL. The scaffolds were placed in a mold. A solution of agarose was poured into the mold. The composite scaffolds were cooled down to allow gelation of the agarose. The samples were dehydrated in a vacuum oven. Rehydration of the scaffolds was done by immersing the dry composites in water and the change of shape was monitored.

RESULTS:Agarose hydrogels dehydrates into films that form random folds. To control the dehydration of agarose hydrogels, PCL scaffolds were embedded in the hydrogel. Notches within the PCL scaffold were added where the folding needed to occur and the bending of the composite was compared between the different designs. After dehydration, we observed that the dry agarose-PCL composites bent exclusively at the notch locations. Because the notches are weak points in the scaffold, stress originating from the agarose drying could concentrate at the notches and bend the composites in a predictable shape. Upon mechanical elongation, the dehydrated composites could resume their flat geometry. We measured the load at break for each designs. The accuracy of the bending was assessed by measuring the angle formed at each notch. To assess the quality of the folding recovery, we conducted several cycles of dehydration-hydration and for each state, we measured the angles around the notch. Finally, tetrahedral triangles designed to fold into a pyramid were manufactured and subsequent rehydration and dehydration lead to a fully closed pyramid demonstrating the potential of this technique to create foldable 3D objects.

DISCUSSION & CONCLUSIONS:This manufacturing process allows to investigate the combination of other materials and create a library of reversible humidity-actuated composites. If scaled down, such foldable composites could be used to create swallowable objects that could deployed under physiological conditions.

References:Foster et al Eur. Polym. J. 117(2019) 159-164

Keywords: Additive manufacturing, Composite materials



Comparative analysis of the results of implantation of decellularized and recellularized pig skin matrices

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INTRODUCTION:The possibility of applying of dermal matrices, which are recellularized with fibroblasts in case of skin damage, makes them promising for wide clinical use for healing wounds with skin surface disorders. The aim of the study was evaluation the results of implantation of acellular and recellularized dermal matrices on a porcine skin wound model.

METHODS:The experiment was carried out on a piglet in the Landrace breed (weight of 25.5 kg). A deep skin wound was formed on the back with a dermatome (15x5 cm). To close the wound surface we used porcine skin matrices, which were decellularized by detergent-enzymatic method with Triton X100 and sodium deoxycholate. Fibroblasts were isolated by enzymatic method and cultured until the 2nd passage. For morphological evaluation of implantation results, a histological study of biopsy samples was performed on the 2nd, 5th, 8th, 16th, 20th day of the experiment.

RESULTS:After implantation in the decellularized matrix and surrounding tissues, an inflammatory reaction developed with a predominance of lymphomacrophagic infiltrate, followed by the formation of scar tissue by 11 days. A study of matrix recellularized samples showed that an inflammatory reaction also developed on days 2-5, however, it occurred only in the surrounding tissues and areas of the matrix bordering the wound bottom. At the same time, perivascular fibroblast proliferation with the appearance of a dermal layer (thickness of up to 200 μm) was noted. On the 8th day under the matrix, the thickness of the newly formed dermal layer increased to 1 mm. The formation of squamous non-keratinizing epithelium with a thickness of up to 5 layers was detected. The upper part of the matrix remained intact, with no inflammation signs. On the 16th day, the thickness of the newly formed dermis reached 2 mm; a large number of newly formed vessels and proliferating fibroblasts were revealed in it. The number of epidermal layers has reached 15, including the stratum corneum. On the 20th day, the number of newly formed vessels and foci of proliferation of fibroblasts and keratinocytes progressively increased. The number of epidermis layers reached 25.

DISCUSSION & CONCLUSIONS:The obtained results showed the presence of reparative changes during closure of the wound surface on a porcine skin wound model by dermal matrices with fibroblast recellularization

Acknowledgements:The work was performed as part of a complex research (№AAAA-A16-116042550089-5 from 25.04.2016)

Keywords: Decellularised matrices, Skin

Morphological analyses of tissue reaction to subcutaneous implantation of decellularized rat heart and lung matrices

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INTRODUCTION:At present time the number of works devoted to the decellularization of various organs has significantly increased. There are many techniques and methods for the preparation of acellular extracellular matrix (ECM), whether based on the physical, chemical and enzymatic effects on the interest. The resulting structure does not contain the saved cells and their decay products, and the complex geometry of the organ, including a relatively intact vasculature, is largely preserved.

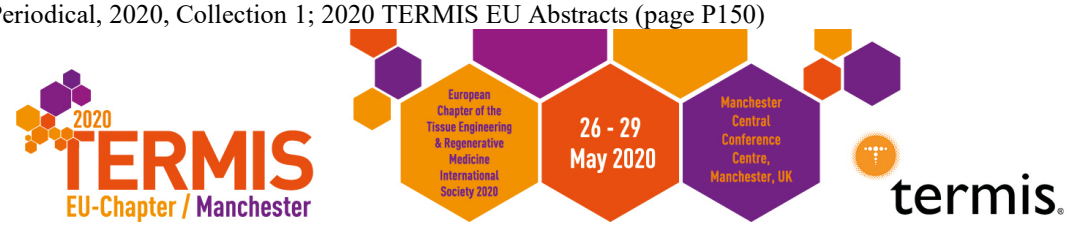
METHODS:The work was performed on 10 male Wistar rats weighing 210 ± 40 g. Decellularization of the lung and rat heart was performed by modified protocols. The samples were implanted subcutaneously in the interscapular region. Rats were derived from experiment on days 7 and 14. We made a qualitative assessment of the composition of the cellular infiltrate around the implant using immunohistochemistry.

RESULTS:Based on the morphological analysis, a histological evaluation of rat tissue response to subcutaneous implantation of the decellularized heart matrices was performed. The qualitative cellular composition of the inflammatory infiltrate was studied with an assessment of the dynamic changes in the macrophages, T- and B-lymphocytes amount on days 7 and 14 after the beginning of the experiment.

DISCUSSION & CONCLUSIONS:The results obtained revealed a different tissue response of the recipient organism to ECM implantation. The least expressed was the response to the lung ECM, which successfully integrated into the tissues and did not undergo significant changes. It is shown that the tissue response to implantation depends not only on the quality of decellularization and the efficiency of antigen molecules removal, but also on the initial histological architectonics and quality of preimplantation preparation of the sample.

Acknowledgements:The study was also supported by the State Assignment of the Ministry of Education and Science of the Russian Federation (project no. 6.5882.2017/BCh).

Keywords: Decellularised matrices, Immunity / immunomodulation / macrophage



Development of a decellularised adipose matrix for tissue repair and reconstruction in the diabetic foot

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INTRODUCTION:Atrophy of the plantar fat pad leads to biomechanical changes in the foot and is associated with the development of diabetic foot ulcers (DFU). An estimated 422 million people were living with diabetes worldwide in 2014 [1] and evidence suggests that 2-3% of patients have an active DFU at any time [2]. Offloading or cushioning through specialist footwear can prevent ulceration, but patients often show poor compliance and a method to directly augment the fat pad at an early stage of degeneration is desirable. Various options have been tested, including silicone injections, acellular dermal grafts or fat grafting [3]. This work describes the development of a decellularised adipose matrix to restore biomechanical function in the plantar fat pad without risk of an immune reaction.

METHODS:A decellularisation process has been developed including low concentration sodium dodecyl sulphate (SDS), nuclease treatment and protease inhibitors, based on a previously established process [4]. Samples of porcine and human subcutaneous fat and porcine infrapatellar fat pad cubes were treated with a range of solvents to remove lipids before undergoing decellularisation.
Process effectiveness was assessed through histology, immunohistochemistry and lipid quantification. Contact cytotoxicity assays were used to assess in vitro biocompatibility of porcine samples.

RESULTS:For porcine adipose, histological staining demonstrated removal of cell nuclei and maintenance of tissue structure following lipid removal and decellularisation. Sudan black B staining showed some residual lipid in samples treated with ethanol or acetone, which was confirmed by quantitative assay; improved lipid removal was seen following isopropanol treatment. L929 cells grew up to samples of decellularised porcine matrix, suggesting it is biocompatible.
Preliminary work on human tissue demonstrates removal of lipids and cell nuclei following isopropanol treatment when samples are less than 5 mm thick.

DISCUSSION & CONCLUSIONS:A decellularisation process has been developed which provides a promising adipose derived scaffold for tissue repair and regeneration. Biomechanical properties will be assessed and solubilisation of the matrix investigated to provide an injectable, off-the-shelf product for plantar fat pad augmentation.

ACKNOWLEDGEMENTS:We would like to thank the donors and their families for the tissue used in this project. The research was supported in part by a Research England Connecting Capability Funded Grow MedTech Proof of Feasibility grant.

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[4] Jones et al., Tissue Eng Part A. 2017; 23(3-4): 124–134

Keywords: Biomaterials, Diabetic healing

The decellularization of human myocardium through novel decellularization tool yields a highly preserved cardiac d-ECM suitable for tissue engineering applications

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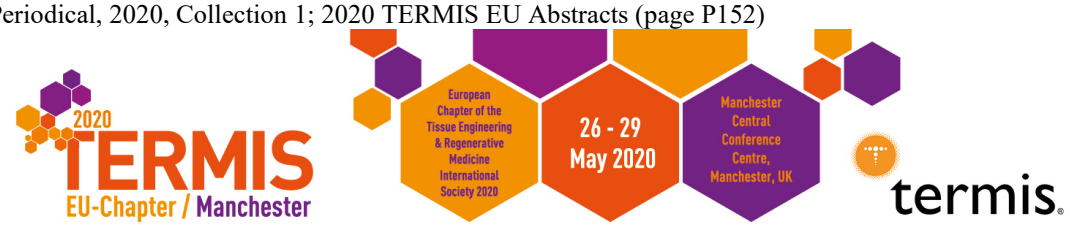
INTRODUCTION:Regeneration of infarcted myocardium represents the main aim of cardiac tissue engineering (CTE). Tissue engineering approach relies on the combination of cells with biophysical stimuli, chemical signals and scaffolds. The scaffold is to provide a template for tissue formation and growth and the ideal scaffold should guarantee a biomimetic tissue-specific behavior to promote and sustain cell homeostasis. Therefore, the choice of the scaffold is a key issue in tissue engineering. Cells in tissues are surrounded by extracellular matrix (ECM), a complex and dynamic network of macromolecules. ECM provides essential physical scaffolding for cellular constituent and sends crucial biochemical and biomechanical cues that are required for tissue and cell morphogenesis, differentiation and homeostasis. Therefore, acellular cardiac ECM appears the most promising platform for ensuring cardiac regeneration. On this basis, aiming at producing a well-preserved cardiac decellularized matrix (d-ECM), we designed an innovative tool to allow simple, fast and effective decellularization of human myocardium.

METHODS:Cardiac samples were harvested from adult human hearts and used to isolate cardiac progenitor cells (hCPCs) or scaled down to fit embedding cassettes and be decellularized by immersion in a solution containing SDS, Triton X-100 and antibiotics under constant agitation. To avoid the uneven solution transport through samples due to random cassette suspension within the beaker, a sample-holder was specifically designed and tailor-made to hold up to four cassettes in a steady position and ensure equal exposure to decellularizing agents. After decellularization, samples were either fixed or snap-frozen and processed for histological, immunohistochemical or molecular biology analyses. hCPC seeded on cardiac d-ECM allowed assessment of cytocompatibility by trypan-exclusion assay.

RESULTS:Hematoxylin and Eosin staining proved the effectiveness of decellularization procedure showing the absence of nuclei. DNA content, resulting well below the accepted threshold of 50 ng/mg of dry tissue, provided further confirmation of decellularization occurrence. Histological analyses documented the preserved three-dimensional architecture of cardiac d-ECM along with the retention of collagen, glycosaminoglycans and elastin that was also confirmed by quantitative dye-binding assays. Additionally, immunohistochemistry revealed in the cardiac d-ECM the retention of proteins typical of native cardiac ECM, like fibronectin, tenascin and laminin, whereas protein array provided evidence of specific growth factor retention, and trypan assay proved the cytocompatibility of the cardiac d-ECM.

DISCUSSION & CONCLUSIONS:These results show that decellularization protocol, combined with a tailor-made sample-holder specifically designed for decellularization procedures under constant agitation, yields a cardiac d-ECM well preserved in its composition and architecture and suitable for CTE applications.

Keywords: Cardiovascular, Biofabrication



Absorption capacity of bone-based carrier-grafts

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INTRODUCTION:In recent years, cell therapy and tissue engineering have become core elements of regenerative medicine. Delivery of cells, antibiotics and expensive growth-factor containing liquids to their intended site of action is difficult as these transferred cells and fluids are easily washed away during surgery and often removed via homeostasis[1]. Future treatments involving cell therapy and tissue engineering products mandate suitable carriers for efficient retention of cells and fluids in situ. In this study, we analysed human tissue scaffolds including high absorption and high surface area carrier-grafts, for their interstitial and matrix-linked absorption capabilities.

METHODS:Eight groups of carrier grafts were analysed comprising two groups of high surface area cortical fiber grafts, demineralized bone matrix (1–2 mm), cortical granules (1–2 mm), three different densities of mineralized cancellous bone and demineralized cancellous bone. All were rehydrated with phosphate-buffered saline (PBS) to a final volume of 0.4 cc. Rehydrated samples were centrifuged in a sieve to separately determine the absorptive capacities of the matrix and interstitial spaces. Total PBS absorption was calculated for all grafts and normalized to the original volume used.

RESULTS:Total absorption – both demineralized cancellous bone (0.844 ± 0.083 ml/cc) and cortical fibers (0.599 ± 0.025 ml/cc) absorbed a significantly larger volume of total PBS compared to the amount absorbed by cortical granules (1-2 mm; 0.376 ± 0.042 ml/cc). Matrix absorption – The largest absorption capacity of the tissue matrix was observed in the cortical fibers (88.82 ± 2.99 %) while low-density cancellous bone displayed the smallest matrix-linked absorption capacity (0.95 ± 1.07 %). Interstitial spaces – Investigating the uptake of PBS into interstitial spaces, revealed that demineralised cancellous bone absorbed the largest amount of PBS into the Interstitial spaces (84.4 ± 3.84 %).

DISCUSSION & CONCLUSIONS:Both cortical fibers and demineralised cancellous bone might provide suitable carrier grafts for regenerative medicine due to their high absorption capabilities. However, matrix absorption is highest in cortical fibers, making these suitable for keeping liquids at the intended target site despite homeostasis. Carrier grafts might provide a valuable addition to the regenerative medicine toolkit.

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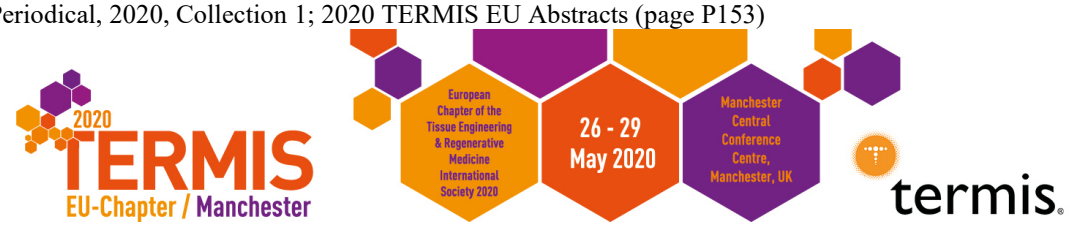
Keywords: Cell therapy, Fibre technology



Physiological mechanical stimulation improves the maturation of d-HuSk-based cardiac bioconstructs in vitro

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INTRODUCTION:Myocardial infarction impairs cardiac integrity affecting the survival and function of cardiomyocytes and causing disruption of extracellular matrix (ECM) architecture and composition.Cardiac tissue engineering (CTE) aims at developing novel approaches to restore the diseased heart combining scaffolds and cells. We have previously demonstrated that decellularized human skin (d-HuSk) provides a cardiac-like environment capable to support survival and differentiation of human resident cardiac progenitor cells (hCPCs) (1). With the present study we investigate the effects of a physiological mechanical stimulation at a frequency typical of either fetal or adult human heart on bioconstructs of d-HuSk repopulated with hCPCs in vitro.

METHODS:To this aim, we cultured hCPC on d-HuSk scaffolds for one week in static conditions. Afterwards, bioconstructs were transferred to a cyclic stretch bioreactor and cultured for three weeks applying a 10% strain at a frequency of 2 Hz or 1 Hz, typical of the fetal or adult human heart, respectively. The effects of described conditions on hCPC engraftment, 3D organization and differentiation were then evaluated by histochemistry, SEM, immunofluorescence and gene expression analyses, using bioconstructs cultured in static conditions as reference.

RESULTS:Histochemical analysis showed that, although hCPCs formed a multilayered tissue on the surface of d-HuSk in both static and dynamic conditions, in constructs cultured in stretch bioreactor cells migrated through d-HuSk to reach its inner layers. From SEM observation emerged that hCPCs in cardiac bioconstructs cultured in static conditions were randomly oriented, whereas hCPC in bioconstructs cultured in stretch bioreactor arranged parallel to each other and orthogonal to the direction of stretch. Intriguingly yet, the membrane localization of connexin-43 in hCPCs stimulated at 2 Hz revealed a more mature phenotype of differentiating myocytes. Gene expression analysis confirmed that physiological cyclic stretch typical of the fetal heart induced the significant up-regulation of genes typical of undifferentiated hCPCs, like CD117, or of early stages of cardiac myocyte differentiation, like TBX-5 and CX37, while a mechanical a stimulation typical of the adult human heart induced the significant up-regulation of late differentiating and mature cardiac myocyte markers, like cardiac myosin heavy chain (MYH7) and connexin-43 (CX43).

DISCUSSION & CONCLUSIONS:Collectively, our results support the evidence that physiological mechanical stimulation of d-HuSk-based cardiac construct boosts the differentiation of hCPCs and strengthen the suitability of d-HuSk as a scaffold for CTE.

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Keywords: Cardiovascular, Bioreactors

Decellularisation of 3D Bioprinted Tissues to Produce Bioactive Implants for Endochondral Bone Tissue Engineering

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INTRODUCTION:The extracellular matrix (ECM) contains numerous regulatory cues which modulate cell fate and tissue development. Decellularised ECMs lacking components that trigger antigenicity or immune responses represent a promising alternative to autografts and allografts for regenerative medicine [1]. Decellularisation of ECM deposited by cells in vitro has gained in popularity since this ECM can be engineered and designed to meet specific requirements. The goal of this study was to test the efficiency of different decellularisation protocols on engineered hypertrophic cartilage with a view to using the resulting matrix for endochondral bone tissue engineering. 3D bioprinting was used to produce engineered hypertrophic cartilage tissues with open architectures to enhance the decellularisation process and produce constructs with architectures compatible with vascularisation.

METHODS:A fibrinogen (3.5 mg/ml) based bioink [2] containing human MSCs ($5x10^6$ cells/ml), was 3D bioprinted and crosslinked. Constructs were cultured in chondrogenic media for 3 weeks at 5% pO_2 followed by 2 weeks at 20% pO_2. Constructs were then decellularised using 4 protocols: (A) freeze/thaw cycles, (B) wash in 0.5% Triton X-100, (C) wash in 0.5% Triton X-100 + 20mM NH_4OH, (D) wash in 0.1% SDS followed by 0.5% Triton X-100. After each protocol, constructs were treated with DNase (10 U/mL). Biochemical assays (DNA, Glycosaminoglycans (GAG) and Hydroxyproline) were performed pre- and post-decellularisation. Histological and immunohistochemical analyses were also undertaken.

RESULTS:The DNA content reduced to 62.4% of its original value for decellularisation method A, and to 32.9% and 24.9% for methods C and D respectively. However, as expected the GAG content reduced to 60.8% for method A and 34.5% for D, but method C retained almost half of the initial GAG (48.6%). Collagen content was less effected by the different decellularisation protocols, reaching a minimum of 78.9% for method D.

DISCUSSION & CONCLUSIONS:These data show that by increasing the harshness of the decellularisation protocol we were able to significantly lower down the DNA content, while maintaining very high levels of collagen and good levels of GAG. Furthermore, the original geometry of the bioprinted tissue is maintained post-decellularisation. We expect that the instructive cues retained in our constructs will allow for better regeneration in vivo. Future studies will explore the potential of these decellularised engineered-ECM templates for promoting the regeneration of large bone defects.

Acknowledgements:Financial support was received from European Research Council (JointPrinting, n°647004) and Trinity College Dublin (Postgraduate Ussher Fellowship).

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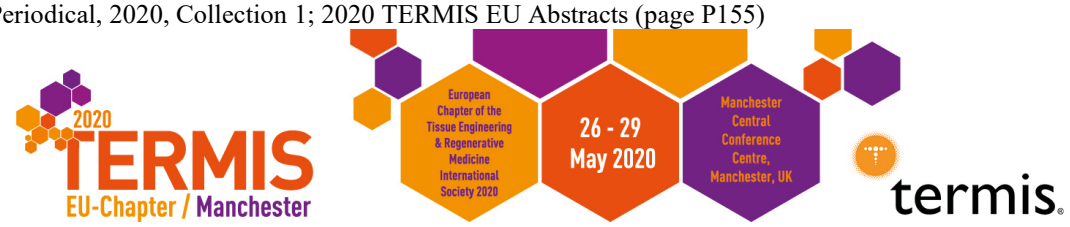
[2] Kang, Nat Biotechnol. 2016; 34(3), 312-319.

Keywords: 3D printing and bioprinting, Bone and bone disorders (osteoporosis etc)



Engineering Tissue-Specific Bioactive Microenvironment from Porcine Extracellular Matrix Hydrogels for Regenerative Medicine

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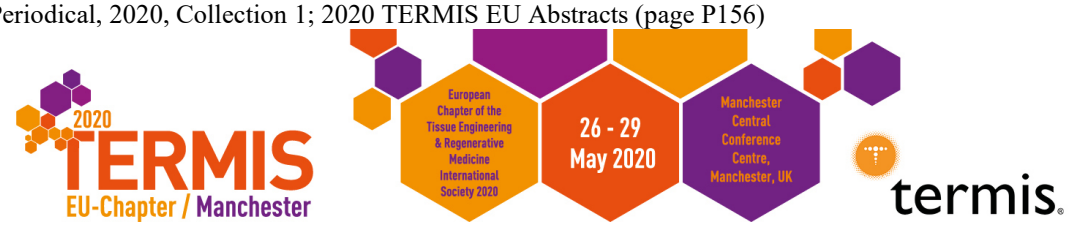
INTRODUCTION:Injectable cell delivery systems for regenerative therapy offer a minimally invasive administration while shielding and retaining the transplanted cells. Furthermore, the use of biomimetic delivery systems can increase cell survival and re-acclimating in the tissue. Due to its unique bioactivity, porcine extracellular matrix (pECM) —the natural bed of cells in the different tissues—holds a great promise as a biomaterial for cell-based therapies. The ECM of each tissue is characterized by a unique composition, organization, and structure developed through the interplay between its different resident cells. These cells secrete molecules affecting the distinctive 3D organization and biochemical profile of the tissue ECM to provide the ideal surrounding suitable for their proliferation, viability, function, and needs.

METHODS:Our aim in this research was, therefore, to develop tissue-specific pECM-based injectable systems for the delivery of various cell types. ECM, isolated from different porcine tissues was enzymatically processed to produce thermally induced pECM hydrogels, which were further assessed to investigate their distinctive properties, and their consequent ability to support cells for biomedical applications.

RESULTS:In terms of composition, each hydrogel had preserved the main collagens and GAGs of its original tissue. Nevertheless, the collagenous profile varied between the different pECM hydrogels leading to microstructural differences in porosity and fiber size distribution. The rheological properties of the hydrogels were also evaluated, revealing soft gel behavior that varied in strength according to the pECM tissue of origin. These hydrogel characteristics highly affected their interactions with cells demonstrated through the different morphology and viability of mesenchymal stem cells (MSCs). Most importantly, the different hydrogels guided a tissue-specific spontaneous differentiation of human induced pluripotent stem cells (hiPSCs), which was proved both by immunostaining and CEL-Seq analyses for tissue unique gene expression.

DISCUSSION & CONCLUSIONS:To conclude, pECM-based cell delivery systems benefit unique, tissue-specific attributes that affect their properties and interactions with resident cells and generate a natural supportive bed for improved cell function and in vivo transplantation outcomes.

Keywords: Hydrogels and injectable systems, Microenvironment and niche engineering



Decellularized Cartilage ECM Reinforced Silk Fibroin Hybrid Scaffolds for Endochondral Bone Regeneration

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INTRODUCTION:An emerging paradigm in the field of bone tissue engineering is to engineer constructs via the endochondral ossification route. Mesenchymal stem cells can be primed towards hypertrophic differentiation, and the primed hypertrophic chondrocytes mediate an enduring pathway for endochondral bone formation. Despite recent success in the endochondral strategy, challenges remain for engineered bone constructs that include functionality under the mechanical environment inside in vivo conditions. We aimed to fabricate a mechanically stable Silk fibroin (SF) carrier incorporating decellularized cartilage derived extracellular matrix (CD-ECM) and hypertrophic chondrocytes as a model of endochondral ossification and characterize superior bone-like tissue formation nondestructively in the case of in vitro studies to find the optimal time point when the constructs should be implanted in vivo.

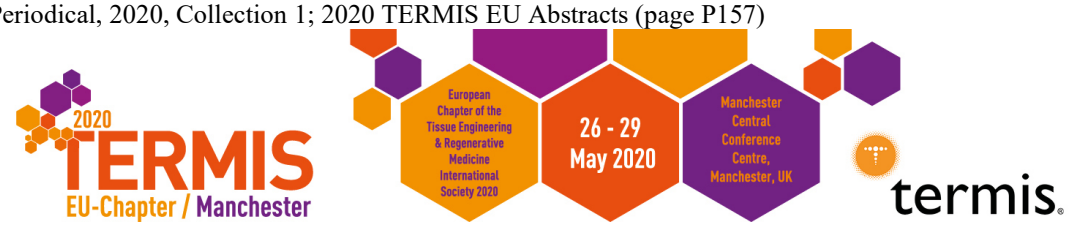
METHODS:CD-ECM/SF scaffolds were produced by combining 17% regenerated silk fibroin with 17% decellularized CD-ECM in HFIP based salt leaching methods with methanol treatment. Human bone marrow stem cells (hBMSC's) were seeded onto CD-ECM/SF or SF constructs and primed 2 weeks for chondrogenesis followed by further 6 weeks of hypertrophy priming in differentiation medium. Biochemical assays, SEM/EDX, RT-qPCR, Biomechanical tests followed by µCT scanning were used as methods at 4 and 8 weeks to determine hypertrophy mediated ossification.

RESULTS:Calcium deposition biochemically determined increased significantly from 4-8 weeks in both SF and CD-ECM/SF constructs and retention of sGAG's were observed only in CD-ECM/SF constructs. SEM/EDX revealed calcium and phosphate crystal localization by hBMSC's under all conditions. Compressive modulus increased by 2.5 fold after 8 weeks of culture in both groups. µCT scanning at 8 weeks indicated a cloud of denser minerals in groups after hypertrophic induction but the BV/TV was higher in CD-ECM/SF constructs than SF constructs. Gene expression by RT-qPCR revealed that hBMSC's expressed hypertrophic markers VEGF, COL10, RUNX2 but the absence of early hypertrophic marker ChM1 and presence of later hypertrophic marker TSBS1 as well as the presence of osteogenic markers ALPL, IBSP, OSX under all conditions.

DISCUSSION & CONCLUSIONS:Our data indicate a new method to prime hBMSC'S into the late hypertrophic stage in vitro in mechanically stable constructs. Incorporation of decellularized cartilage derived ECM onto a mechanical stable silk fibroin scaffold promotes the endochondral ossification route bone tissue regeneration.

Acknowledgements:Funding by Life science calls LSC16-024 from NÖ Forschungs- und Bildungsges.m.b.H (NFB) and the provinical government of Lower Austria.

Keywords: Decellularised matrices, Biomaterials



Development of porcine xenograft-derived diaphragmatic decellularized extracellular matrix scaffolds for Congenital Diaphragmatic Hernia repair: toward the clinical translation

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INTRODUCTION:Congenital diaphragmatic hernia (CDH) occurs during prenatal development, when the diaphragm fails to fully form allowing herniation of abdominal organs into the thoracic cavity. This leads to partial failure in lungs and hearth development. An innovative approach for the repair of this congenital defect is the use of a portion of decellularized diaphragm extracellular matrix (dECM) to close the large muscle defects, as alternative solution to the current gold standard treatments (e.g. synthetic patches). Recently we have demonstrated the advantages of using dECM in vivo in a CDH mouse model and that dECM can be used as scaffold for the generation of an engineered diaphragm in vitro. Here we translate our approach to generate clinical scale porcine-derived dECM as relevant acellular xenograft scaffold for future clinical translation.

METHODS:We are investigating different strategies (perfusion/diffusion) for the decellularization of porcine diaphragm that will allow us to use this scaffold for different purposes. From one hand, the perfusion of reagents through the muscle vascular tree will allow us to use this scaffold as intact acellular patch; from the other hand, diffusion decellularization obtained upon fragmentation of the original diaphragm would be useful as starting material for in vitro specific preclinical studies and as ECM-derived bioink for 3D muscle regeneration. We investigated the efficiency of sterilization (peracetic acid /gamma irradiation) and storage procedures. Alpha-Gal quantification was tested to assess xenograft safety and potential future clinical translation.

RESULTS:We have characterized both the decellularization METHODS: histological, electron microscopy and biochemical validation confirmed successful removal of cells and DNA, as well as the preservation of native ECM components and structural architecture. Moreover, we performed pilot experiments of scaffold repopulation using human muscle precursor cells to explore the possibility of creating a xenograft-derived humanized muscle. Starting from Piglet dECM we also generated a specific bioink in order to explore a different approach to fabricate a 3D bioprinted diaphragm.

DISCUSSION & CONCLUSIONS:We propose here different strategies to generate a new clinically relevant approach for CDH repair, based on porcine decellularized diaphragmatic ECM. We investigated clinical needs, production steps and validation procedures (xenograft characterization, biocompatibility, sterility, storage) to generate a biomedical device that would translate our research into clinic.

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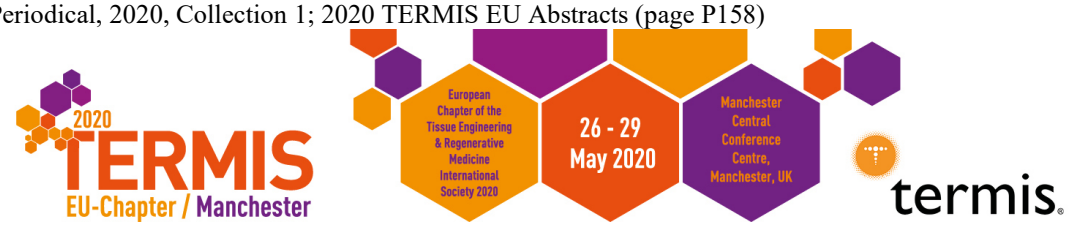
Keywords: Decellularised matrices, Musculoskeletal (inc ligament / tendon / muscle / etc)



Tissue engineered lung membrane patches

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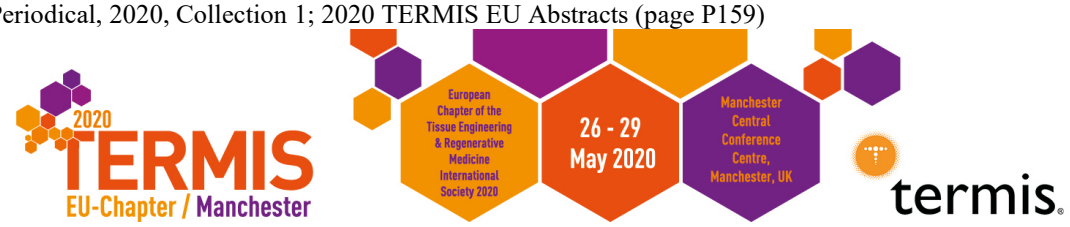
INTRODUCTION:Prolonged pulmonary air leaks following lung resection surgery is a source of patient morbidity and mortality, with a post-surgical incidence of 8%-26%. Current clinical practice employ conservative management strategies through chest drainage and chemical pleurodesis. Tissue engineered pleural patches could be a therapeutic alternative to clinical management of pulmonary air leaks, with the ability to repair and restore injured pleural membrane (PM) structure and function. Decellularised PM as biologic scaffolds offer an advantage over fabricated scaffolds retaining native microarchitecture and extracellular matrix (ECM) composition while reducing the risk of immune-intolerance, with the removal of cellular and nuclear material. This study aimed to develop a reliable decellularisation protocol for PM.

METHODS:PM was excised from freshly sourced pig lungs. The excised membranes were subjected to a decellularisation process initiated by a freeze-thaw cycle followed by a treatment with a decellularizing buffer containing 1% Triton X-100 and 0.5% Sodium deoxycholate. The treated pleural samples were studied and compared to their native counterparts through histology staining and quantification, (H&E, Alcian blue and Picosirius red), nuclear DNA integrity evaluation (DAPI), PM thickness and estimation and mechanical testing.

RESULTS:H&E staining confirmed the absence of nuclei in the decellularised PM suggesting removal of cellular and nuclear material. Structural organisation and alignment of the treated membranes were comparable to the native PM. Alcian blue staining (sulphated glycosaminoglycans) and Picosirius red (collagen) indicated retention of the native membrane composition and structure with no obvious alterations following on from decellularisation. Quantification of DAPI-stained nuclei indicated an absence of nuclei in the decellularised PM in comparison to the native PM ($p<0.0001$). Average membrane thickness of the decellularised PM was 218.33 ± 67.64µm as opposed to native PM measured at 145.31 ± 33.07µm. Mechanical characterisation determined the average Young's modulus of native and decellularised PM with no significant difference, at values of 828.44±177kPa and 804.08±670.49kPa respectively.

DISCUSSION & CONCLUSIONS:Histological and mechanical evaluation of decellularised PM indicates the development of a successful strategy to remove cellular and nuclear material with minimal disruption to native structural composition, alignment and mechanical characteristics. This represents a first step towards providing a clinical alternative to current, sub-optimal, therapeutic approaches for the treatment of pulmonary air leaks.

Keywords: Cell therapy, Wound healing



Development of a protocol for the production of dermal extracellular matrix

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INTRODUCTION:Statement of the Problem: Despite the achievements of modern surgery in the treatment of cutaneous injuries, the search for new methods for faster and more effective wound healing remains topical. Tissue engineering is undoubtedly of interest for the development of such technologies.Aim of the study was to determine the optimal protocol for obtaining a decellularized dermal matrix for the subsequent development of tissue-engineered skin.

METHODS:The experimental animal was 1 pig of the Landrace breed. After skin pretreatment with a dermatome, the samples were taken of a thickness of 0.3 cm. Two decellularization protocols were examined: protocol No.1 based on the using of Triton X-100 and deoxycholate, protocol No.2 only based on deoxycholate. Total processing cycles for 2 protocols were 5. The acellular matrices after treatment were examined with: histological analysis, quantitative determination of DNA content in wet tissue. Further, static matrix recellularization of porcine dermis fibroblasts was carried out. After that, the matrices were assessed for cytotoxicity using an XTT test and a test for the differential staining of living and dead cells.

RESULTS:The comparative analysis of two protocols for decellularization of porcine dermis showed that both protocols effectively remove cells and nuclear material, while maintaining the architectonic of the intercellular substance intact, since fibrous structures are not destroyed. But when analysed the functional properties of matrices on the basis of cell viability analysis according to the XTT test and cell adhesion to the matrix, the matrix processed according to protocol No.1 demonstrates the advantages.

DISCUSSION & CONCLUSIONS:In this study, a decellularization protocol based on Triton X-100 and deoxycholate was noted. The results are the first stage for the further development of tissue-engineered skin.

Acknowledgements:This study was performed as part of the complex research program of the Laboratory of Basic Research in Regenerative Medicine of the Medical University of the Ministry of Health of the Russian Federation “Cellular Mechanisms of Regeneration of Intrathoracic Organs and Tissues. Development of tissue engineering structures using biological and synthetic scaffolds"

Keywords: Decellularised matrices, Skin

Development of a composite decellularised osteochondral scaffold for the treatment of articular cartilage lesions

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INTRODUCTION:Autologous chondrocyte implantation has recently emerged as a promising regenerative approach for cartilage repair and achieved NICE accreditation for the treatment of lesions >2cm² [1]. However, prior to regeneration the cell/matrix graft has limited mechanical strength or function, and has complications such as cell leakage [2]. We propose the use of cell seeded decellularised osteochondral grafts as a biomechanically relevant treatment for cartilage defects. This work details development and assessment of a decellularised scaffold of dimensions appropriate for large shallow lesions, initial viability results of C20A4 chondrocyte cells encapsulated in a self-assembling peptide (SAP) cell delivery system, and attempts at delivering cells to the decellularised scaffolds.

METHODS:Porcine femoral condyles were shaped to dimensions of 2cm² [3] with a depth of 5mm and subject to the University of Leeds decellularisation process [4]. These scaffolds were characterised qualitatively for decellularisation with H&E, and quantitatively via a total DNA assay. A contact cytotoxicity assay with L929 and BHK cell lines was also conducted. These scaffolds were subsequently seeded with C20A4 cells, via static, vacuum and injection seeding, and assessed for cell viability, distribution and penetration. Finally, biocompatibility of a SAP-GAG hydrogel was assessed with L929 cells over a 14-day period with alamarBlue and LIVE/DEAD viability measures.

RESULTS:Scaffold characterisation revealed cell removal, <50ng/mg total DNA and confirmed scaffold cytocompatibility. For the attempts at recellularisation, static seeding showed the most homogenous seeding pattern from LIVE/DEAD staining, however, cells barely penetrated the superficial layer. Cell delivery in a SAP-GAG hydrogel was also investigated. Initial experiments with fibroblast cell lines indicate an ability to support cell proliferation over a 14-day period.

DISCUSSION & CONCLUSIONS:This work provides promising indications for the production of a biomechanically relevant matrix for cell implantation and cartilage repair. Future work will investigate methods of improving cell penetration into the cartilage matrix, as well as long-term culture experiments to study the remodelling response in terms of ECM deposition and restoration of mechanical properties.

Acknowledgements:Dr Hazel Fermor
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Dr Louise Jennings
Dr James Warren

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Keywords: Decellularised matrices, Knee

Novel 3D Embryo Implantation Model within Macroporous Scaffolds

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INTRODUCTION:Implantation failure remains an unsolved obstacle in reproductive medicine and is a major cause of infertility in otherwise healthy women. Previous studies suggested that the adhesion molecule E-cadherin played a key role in the implantation process [1]. Due to obvious ethical limitations, there is an unmet need to establish an in vitro model that mimics the events in the uterine wall during the implantation process. The available in vitro two-dimensional and hydrogel-based models demonstrate short-term endometrial cell culture under hormonal treatment. Our objective was to examine endometrial cell culture in highly porous scaffolds for 3 weeks under hormonal treatment, mimicking the menstrual cycle.

METHODS:Alginate scaffolds were prepared by a freeze-dry technique [2]. Epithelial endometrial were seeded and cultured for 3 weeks under hormone treatment that mimicked a typical menstrual cycle. Cell constructs were evaluated for E-cadherin mRNA expression levels by qPCR and for E-cadherin protein expression by specific immuno-staining and Western blot analysis. Human choriocarcinoma (JAR) spheroids, utilized as embryo-like tissue models, were seeded onto three-week old cell constructs, and their attachment to the 3D tissue model was examined by H&E staining, as a measure of endometrial tissue receptivity.

RESULTS:The epithelial tissue-like constructs of the receptive human endometrial RL95-2 cell line were responsive to hormones, as judged from the hormone-dependent E-cadherin expression levels; and most importantly, were functional in terms of their ability to adhere to JAR spheroids, mimicking the first step of implantation. A parallel 3D model of human endometrial HEC-1A cells, expressing lower levels of estrogen receptor alpha (ERα), was established as a second model of non-receptive endometrium tissue, partially mimicking an endometrium of in vitro fertilization (IVF) patients suffering from recurrent implantation failure (RIF). The HEC-1A model demonstrated lower levels of E-cadherin and JAR spheroids were not able to attach to it. Forced ERα overexpression in HEC-1A cells restored E-cadherin expression levels and rescued JAR attachment capability.

DISCUSSION & CONCLUSIONS:The proposed 3D model of endometrial cells within macroporous alginate scaffolds enabled long-term culture of viable endometrial cells. The established model can serve as an important tool for elucidating the regulatory mechanism of the implantation process, and for screening and evaluating potential novel therapeutic strategies for repeated implantation failure.

Acknowledgements:Azrieli College of Engineering, Jerusalem (DS).

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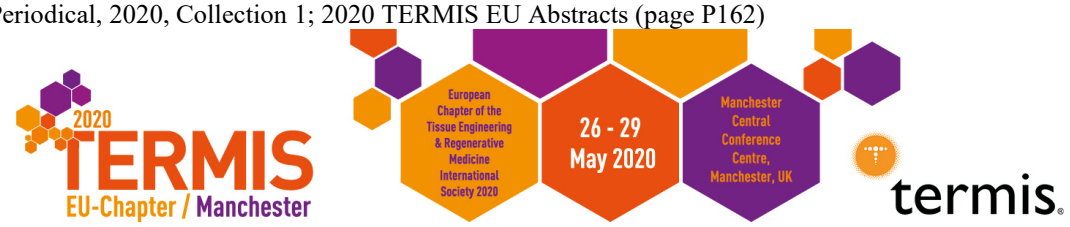
Keywords: Developmental biology,



The proangiogenic Thrombospondin-4 is produced by hypertrophic chondrocytes during endochondral bone formation

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INTRODUCTION:Endochondral ossification (EO) is the process by which transient cartilage templates are formed and replaced by bone. It takes place during skeletal development and bone fracture healing. In order to produce bone, different tissue engineering strategies aim to replicate EO using mesenchymal stem cells (MSCs). One essential step during EO is the vascularisation of the transient cartilage, which requires instructive signals for the induction of blood vessel ingrowth. The ECM protein thrombospondin-4 (TSP-4) is a candidate. TSP-4 is described to play a role in angiogenesis, and is present during both MSC in vitro chondrogenesis [1] and epiphyseal cartilage development in vivo [2]. Here we study the spatiotemporal location of TSP-4 during EO to obtain more insight in its role and a possible link with vascularisation.

METHODS:Epiphyseal plates were obtained both from explanted digits of 1-2 year-old human patients affected with polydactyly, and from mouse tibiae at different times of postnatal growth: E0,7,14,21,28,64. TSP-4 localisation was assessed by immunohistochemistry. The angiogenic effect of 10 µg/mL recombinant TSP-4 was assessed in vitro using human umbilical vein endothelial cells (HUVECs). HUVEC proliferation over 24h was investigated with the EdU cell proliferation kit. HUVEC migration towards TSP-4 was assessed in a modified Boyden chamber assay after 10h.

RESULTS:TSP-4 immunohistochemical staining localised the protein at the hypertrophic zone of the epiphyseal plates, both in human and in mouse. TSP-4 signal was mainly detected at the pericellular matrix of hypertrophic chondrocytes, which was lost upon contact with the ossification front. During growth, mouse hypertrophic chondrocytes, both from the epiphyseal plate and the nearby secondary ossification center, consistently produced TSP-4. While TSP-4 did not affect HUVEC proliferation in vitro, it induced a 7-fold increase on their migration ($p<0.05$).

DISCUSSION & CONCLUSIONS:Previous research has detected the presence of TSP-4 on mouse epiphyses, but not its location. In our study, by using a validated antibody we have observed TSP-4 in both human and mouse growth plates. Our results indicate that TSP-4 is produced by hypertrophic chondrocytes during developmental EO. Because of its proangiogenic function, TSP-4 may contribute to the ingrowth of blood vessels into the cartilage matrix. Further research should focus on its potential use for bone tissue engineering purposes.

ACKNOWLEDGEMENTS:Funded by European Union's H2020 MSCA-ITN grant No.721432.

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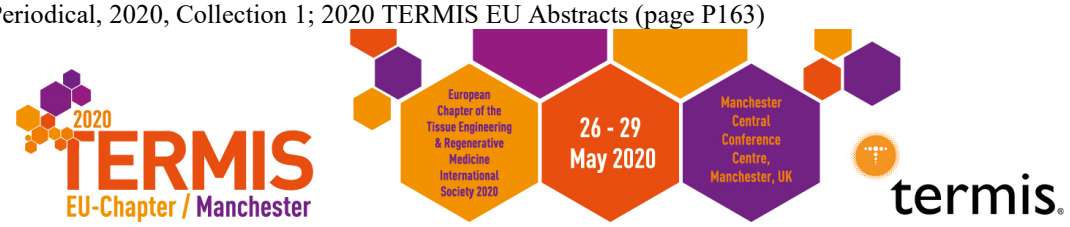
Keywords: Cartilage / joint and arthritic conditions, Bone and bone disorders (osteoporosis etc)



Gonadotropin-free culture condition for ovarian primordial follicles

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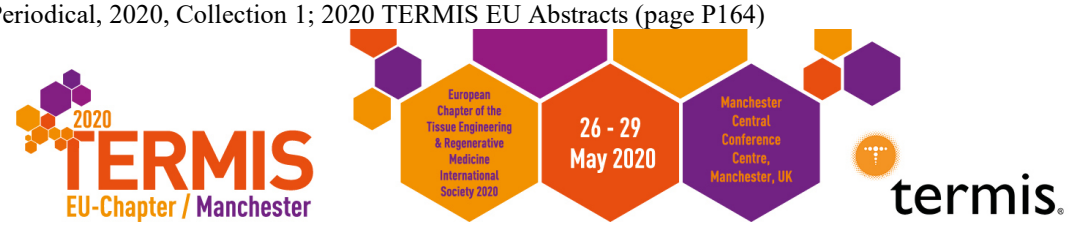
INTRODUCTION:Ovarian primordial follicles are the fundamental of ovarian reserve and the longest residual population in reproductive span of women. According to this perspective, primordial follicles are considered as germ cell specific cell source for fertility preservation, however, their optimal in vitro follicular maturation (IVFM) condition or in vivo generation mechanism have not been revealed. In this study, in vitro culture condition of Gonadotrophin-free primordial follicles are established and evaluated.

METHODS:Ovaries from 13-day-old B6C3F1 female mice were dissected and primordial follicles were isolated. Isolated primary follicles were cultured in the absence or presence of bFGF. The IVFG into secondary follicles were observed and the gonadotropins were added to induce maturation into Graffian follicles. The expression of specific genes of each follicular stage was analyzed using qRT-PCR.

RESULTS:Gonadotropin-free, in vitro follicular maturation of primordial follicles were conducted using bFGF with MEM alpha medium. The follicles were matured into secondary follicles by 10 days and the existence of FSH receptor was confirmed. Furthermore, the follicles were successfully ovulated MII oocytes.

DISCUSSION & CONCLUSIONS:Conclusively, gonadotropin-free IVFM condition for ovarian primary follicles was successfully established. This result could be applied for the further use of primordial follicles as cell source of fertility preservation (2016R1D1A1B03934784 and 2016R1E1A1A01943455).

Keywords: Developmental biology, Cell therapy



Generation of human embryonic and fetal pancreatic organoids as a model of pancreas development

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INTRODUCTION:Diabetes mellitus (DM) represents a significant global health and economic burden, and its incidence is increasing. Currently 463 million people have diabetes worldwide; this number is estimated to increase to 700 million by 2045[1]. Although injectable insulin is the gold standard for Type 1 DM management, it confers life-long dependence and hypoglycaemia persists in patients. Therefore, restoration or regeneration of damaged insulin-producing β-cells could provide a potential cure. However, to do this safely a greater understanding of β-cell specification during early human development is required. Organoid systems refer to clusters of 3-dimensional (3D) self-organising epithelial cells that can mimic the cell-cell contacts and signalling events of the native tissue environ[1]ment. Human fetal pancreatic organoids are emerging as exciting models for pancreas development, enhancing our understanding of β-cell differentiation. This project aims to establish a 3D organoid model of human pancreas development and regeneration from human embryonic and fetal pancreatic progenitor cells (PPCs).

METHODS:Human embryonic and fetal pancreata were dissected and dissociated into single cell suspension before incubation with Matrigel. Organoids were cultured for up to 30 days before analysis. Following incubation, organoids were assessed by immunohistochemistry and qPCR. For comparison, immunohistochemistry and light sheet fluorescence microscopy (LSFM) was performed on native embryonic and fetal pancreata.

RESULTS:Immunohistochemistry reveals the spatial and temporal expression profiles of key pancreatic transcription factors (TFs) in embryonic and fetal pancreata, with distinct 3D ductal and islet structures observed in fetal samples using LSFM. The expression of these TFs characterises our human fetal pancreatic organoids. PPC markers, NKX6.1, SOX9 and PDX1, and ductal cell marker, CK19, are detected.

DISCUSSION & CONCLUSIONS:Pancreas forms when foregut endoderm is specified to pancreatic endoderm, followed by PPCs and then acinar or bi-potent ductal and endocrine populations, including β-cells. 3D organoid culture of fetal pancreas permits expansion of multipotent progenitors, which can be used as a platform for modelling downstream endocrine differentiation. Future steps include the manipulation of the surrounding niche and growth factor environment to study the effects of endocrine (and β-cell) differentiation, as well as comparison with pluripotent stem cell- (PSC-) derived pancreatic organoids. Gene editing techniques will also be used to induce the expression of endocrine or β-cell specific genes. In summary, the establishment of this pancreas organoid system is a valuable asset for furthering our knowledge of pancreas development and endocrine commitment.

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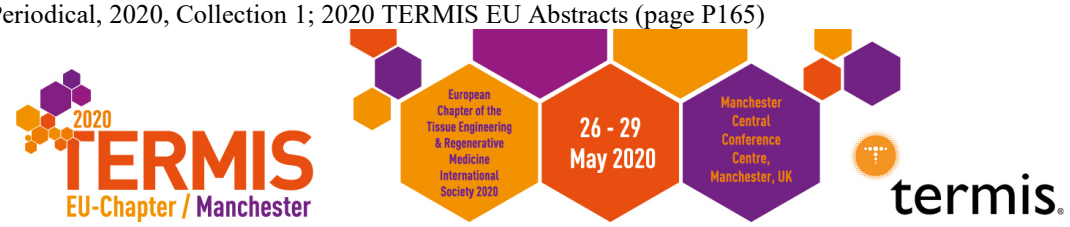
Keywords: Differentiation, Cell therapy



Potential of biogenic ions doped biodegradable inorganic nanofibres as wound healing promoter in diabetic wounds

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INTRODUCTION:Recently, diabetic wound management, nutrition and drug delivery have attracted significant attention leading to evolution of nano-based dosage forms and wound pads for active wound management of contaminated and slow-to-heal wounds. Among all types of nanomaterials, nanofibres have shone due to their unique properties leading to barrier effect and increased drug bioavailability. In addition to these properties held by nanofibres in general, the chemical nature is a crucial parameter, possibly able to modulate wound healing process through molecules released into the wound site. From this point of view, silica nanofibres represent a very promising material enhancing traditional nanofibres properties by their inorganic nature itself as the silicic acid, released during their degradation, is well known for its wound healing stimulating potential and can be supported by bioactive compounds incorporation. In this initial study, we evaluated impact of biogenic ions of Zn+, Ca2+ and Cu2+ incorporation on wound healing in normal and diabetic environment.

METHODS:The silica nanofibres were obtained by modified sol-gel synthesis method from the TEOS precursor supplemented by bioactive compounds and subsequent electrospinning. Effects of the ions on wound healing process were studied on normal human dermal fibroblasts (NHDF) and diabetic patient harvested dermal fibroblasts (DHDF). Impacts on proliferation, motility in wound scratch assay and ROS stress were studied. Impact on angiogenesis was studied on HUVEC cells by tube formation assay.

RESULTS:The biogenic ions doped silica nanofibres exhibited quick ion release related to the unique corrosion-like degradation of silica nanofibres. The first ions were released within the first six hours of exposure and continued for 72 hours minimum. Presence of Ca2+ and Cu2+ led to increase of proliferation and motility of NHDF and DHDF. Zink ions proved capacity of oxidative (ROS) stress reduction in DHDF in DCFDA assay. Proangiogenic effect of Cu2+ ions was observed in all three tested concentration after 48 hours of exposure.

DISCUSSION & CONCLUSIONS:Bioactive ions doped silica nanofibres were confirmed as a biocompatible, biodegradable ion eluting material with a potential for application in diabetic wound management. Incorporation of the selected ions - calcium and copper - led accelerated wound closure and improved angiogenesis. Zink presence led to modulation of oxidative stress in DHDF.

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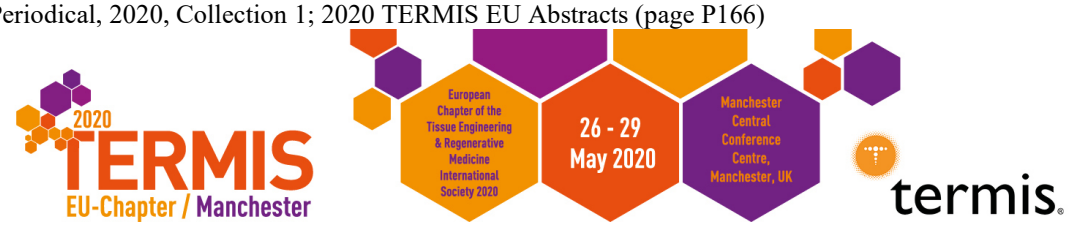
Keywords: Nanomaterials (inc graphene), Diabetic healing



Growth and differentiation of iPSC-derived kidney organoids using fully synthetic peptide hydrogels

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INTRODUCTION:Induced pluripotent stem cell (iPSC)-derived kidney organoids have been shown to closely recapitulate fundamental tissue developmental processes and represent a useful method to examine aberrant gene regulatory mechanisms that occur in diseases such as Diabetic Nephropathy. Despite significant progress made towards understanding signalling mechanisms and refining organoid models over the last number of years (1,2), there is a growing necessity to increase complexity, reduce variability and improve maturity and genetic authenticity. We propose that organoid development will benefit from 'tunability' of the biophysical properties of the cellular microenvironment. Synthetic hydrogel scaffolds represent a promising alternative to circumvent limitations associated with tissues grown on traditional, naturally-derived extracellular matrices. These scaffolds are also more amenable to generating mechanical and signalling gradients that more accurately mimic the in vivo environment.

METHODS:We differentiated iPSC-derived nephron progenitors into kidney organoids within fully defined, self-assembling peptide hydrogel scaffolds of variable stiffness and component peptide charges. Organoids were characterised using immunohistochemistry and histological staining methods.

RESULTS:Transmission electron microscopy of hydrogels revealed a fibrous structural architecture similar to that of matrigel like-extracellular matrices. Haematoxylin and Eosin staining of encapsulated organoids exhibited variable morphological self-organisation of epithelial cell tubular structures within hydrogels by day 24, particularly between highly charged peptide hydrogels with Young's Moduli of 1 and 14 kPa. Interestingly, immunofluorescent characterisation also showed differential proximal (LTL+ve) and tubular epithelial (ZO-1+ve, ECAD+ve) expression between the embedded organoids. Additionally, we have identified distinct combinations of extracellular matrix components, including vitronectin and laminin-511, that support differentiation of iPSCs towards renal lineages in two-dimensional culture. Cellular self-organisation into LTL+ve and ZO-1+ve structures accompanied by laminin basement membrane formation was observed by day 18 of differentiation. Functionalisation of these substrates within peptide hydrogels will likely further enhance processes of renal differentiation.
DISCUSSION & CONCLUSIONS:To the best of our knowledge, this is the first investigation of iPSC-derived kidney organoids within fully synthetic, self-assembling peptide hydrogels. These results will further support the use of designer matrices that will improve iPSC differentiation towards renal cell fate trajectories.

ACKNOWLEDGEMENTS:This work is supported by Science Foundation Ireland and the Curam Research Centre, in partnership with Manchester Biogel.

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Keywords: Hydrogels and injectable systems, Kidney

Engineered in vitro model for breast tumour progression: pH abrogates effects of matrix stiffness and fluid flow on membrane marker expression

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INTRODUCTION:Multiple biophysical factors like tissue stiffness, oxygen content, pH and interstitial fluid flow influence tumour progression and development. Traditional cell culture models lack physiological complexity and don't reflect variation of biophysical stimuli; whereas, with animal models these biophysical variables cannot be controlled. Here we show how engineered in vitro models can be used to better represent tumour progression [1]. These consist of a three-dimensional cell cultures model included in a microfluidic perfusion system, providing the advantage of accurate control over biophysical stimuli and engineer tissue pathophysiology at a micro-scale. We focused on stiffness and pH variation, as well as perfusion to study changes in proliferation and membrane marker expression for stemness in invasive breast cancer cell line.

METHODS:MDA-MB-231 breast cancer cells were cultured with one or a combination of biophysical stimuli, i.e. stiffness, pH, fluid flow. Cell proliferation and viability were quantified by Alamar blue assay, whereas marker expression (CD44, CD24, CD44v6) was assessed by flow cytometry over two weeks.
Matrix stiffness: Hydrogels were designed to mimic different matrix stiffness and adhesion ligand density (LD) ranging from 1-10kPa, similar to in vivo breast tissue and tumour stiffness [2]. Cells were encapsulated and cultured within alginate|gelatin hydrogels. Dynamic culture: Ibidi pump system was used to perfuse MDAMB-231 cells with 0.5mL/min of media flow (shear stress: ~0.13dyne/cm2).
pH: Cells were cultured in complete cell culture media buffered at pH values of 6.5 (tumour) and 7.4 (healthy).

RESULTS:We observed a reduced proliferation of MDA-MB 231 in all the engineered models compared to traditional 2D model. Perfusion and lower pH value increases the expression of both CD44 and CD44v6; whereas the combination of fluid flow and low pH value results in an increase in CD24 expression. Additionally, increase in stiffness is proportional to the expression of CD24. However, at low pH the correlation between hydrogel stiffness and CD24 expression is attenuated.

DISCUSSION & CONCLUSIONS:We here report how changes and interplay of biophysical stimuli influence breast cancer cells. Marker expression for CSCs is still under debate [3], hence further functional assays are needed to understand implications of these biophysical variations. Nonetheless, these models are a starting point to further understand metastatic potential and its molecular mechanisms.

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Keywords: Biomechanics / biophysical stimuli and mechanotransduction, In vitro microenvironments

Patient-derived innervated 3D skin model as novel in vitro tool for the investigation of small fiber neuropathy

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INTRODUCTION:Small fiber neuropathy (SFN) is characterized by acral burning pain and dysesthesias. SFN primarily affects thinly myelinated A-delta and unmyelinated C fibers responsible for pain and thermal perception. The underlying mechanisms of SFN are unknown and research is hampered by the limited availability of biomaterial restricted to few mm² of diagnostic skin punch biopsies. After having established primary fibroblast and keratinocyte cell cultures, our aim is to generate innervated three-dimensional (3D) in vitro skin models entirely produced using patient-derived skin cells and induced pluripotent stem cell (iPSC)-derived neurons. These personalized innervated skin models will be applied as novel in vitro tools to individually investigate the pathophysiology of SFN.

METHODS:Fibroblasts and keratinocytes were isolated from diagnostic skin punch biopsies and 3D in vitro skin models were generated. The histological structure of 3D skin models was assessed using immunohistochemistry. 3D skin models were innervated using patient-derived sensory neurons which were generated via induced pluripotent stem cells from the fibroblasts of the same skin punch biopsy. For cellular characterization, expression of neuronal and peripheral marker proteins was analyzed using immunocytochemistry.

RESULTS:We established primary cell cultures of fibroblasts and keratinocytes from skin punch biopsies of SFN patients, and also succeeded in generating 3D skin models displaying regular human skin morphology as an in vitro tool for pathophysiology research of SFN. First innervated patient-derived 3D skin models showed the expression of peripheral markers and keratinocyte specific proteins. The histological structure as assessed by HE staining was normal.

DISCUSSION & CONCLUSIONS:We provide an entirely human, personalized 3D innervated in vitro skin model that will open new avenues for in depth pathophysiological investigation of SFN and will revolutionize the pre-clinical assessment of novel analgesic compounds.

Acknowledgements:The project is funded by the Interdisciplinary Center for Clinical Research (IZKF, N-353) of the University of Wuerzburg.

Keywords: Nervous system (brain-central-peripheral / disorders), Skin

Development of serum-free, defined media for the setup of an inflamed adipose tissue model

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INTRODUCTION:Inflamed adipose tissue is associated to different pathologies like type 2 diabetes or crohn's disease [1]. In vitro models of inflamed adipose tissue could help to gain insights into the development of the pathological conditions. Additionally, the screening for potential drugs, which target related diseases, could substantially benefit from corresponding models. Most current tissue engineering approaches depend on the use of animal-derived serum. The use of serum is however linked to high batch-to-batch variations and ethical concerns [2,3]. In this study, we aimed to set up an inflamed adipose tissue model in vitro based on fully mature adipocytes (MAs). In order to avoid potential interferences, we aimed to develop defined adipocyte and monocyte culture media for the serum-free setup of the model.

METHODS:MAs were isolated from human subcutaneous adipose tissue and encapsulated in 3D hydrogels based on collagen type I. Defined adipocyte culture media were developed and compared to the serum-containing control during a 14-day culture period. Adipocytes' phenotype was analyzed based on the expression of perilipin A and the release of leptin. The proliferation behavior of the human monocytic cell line Monomac6 (MM6) was analogously tested in defined and serum-containing conditions. Further, the activability of the monocytes under defined conditions was analyzed based on the secretion of pro-inflammatory cytokines like Il-6, Il-1β and TNF-α. Adipocyte-containing hydrogels and MM6 were combined to set up a co-culture system of inflamed adipose tissue.

RESULTS:A defined adipocyte culture medium was developed, which supported the maintenance of the adipogenic phenotype up to 14 days. Proliferation and activation of MM6 comparable to the serum-containing control culture was confirmed using an adapted defined culture medium. Based on the developed media a co-culture model of MAs and monocytes was successfully established. Furthermore, the maintained activability of monocytes was proven in the co-culture setup.

DISCUSSION & CONCLUSIONS:The developed defined media allow the setup of a co-culture model including maintained characteristic MAs and the activability of monocytes. Based on these achievements, patient-specific inflamed adipose tissue models could be set up to investigate the underlying mechanism of different pathological manifestations and analyze drug response individually.

Acknowledgements:Financial support was received from the Ministry of Science, Research and Art Baden-Wuerttemberg and the European Commission (#1099895).

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Keywords: In vitro microenvironments

Functional tissue-engineered healthy and pathological "in vitro" models to study osteoarthritis

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INTRODUCTION:Osteoarthritis (OA) is a degenerative pathology that affects large size of population and leads to significant impaired of the quality of life and disabilities. Joint OA is characterised by destruction of the articular cartilage (AC), inflammation of synovial membrane and remodelling of the subchondral bone [1;2]. The increased expression of catabolic proteins by synoviocytes, chondrocytes and mononuclear cells ensues by action of stimuli such as cytokines or chemokine [3]. Several studies have been carried on deepening the knowledge on the onset of OA and its treatment, but there are still uncertainties about a long-term surgical treatment for OA joints [4]. The aim of this work was to develop an engineered in vitro models of healthy and osteoarthritic AC human tissues in order to increase our understanding mainly on physiology, biology, and progression of OA diseases. The findings of this work can advance the knowledge and understanding of the causes of the OA disease as a key tool for testing new therapeutic options.

METHODS:For the subchondral bone, a polylactic acid (PLA) porous structure was 3D printed and functionalized with gelatin (Gel) and hydroxyapatite (nHA) through immobilisation via poly-dopamine (DP) coating. While for the cartilage deep zone, a photocurable Gellan Gum (GG; 3%w/v) hydrogel structure with channels was obtained via soft-lithography and attached to the 3D PLA scaffold before the GG crosslinking. Chondrocytes (differentiated from Y201 bone marrow stromal mesenchymal stem cells) [5] were encapsulated in each channel of GG layer within a chondroitin sulphate-based hydrogel, while Y2O1 cells were seeded on PLA scaffold. OA milieu was induced by IL-6, IL-1β and TNF-α addition to the medium.

RESULTS:Construct water uptake capability (initial uptake 1,433±57% at 3h, then plateau was achieved), high porosity (30% pores 100-150μm in diameter) and suitable mechanical properties (48kPa±5kPa Young's modulus) were assessed. All the materials were cytocompatible in terms of cells viability and metabolic activity up to 7 days. The effects of cytokines inducing OA decreased the mechanical properties with an increase of the OA-related molecule expression for the pathological model (CollI, MMP9-13, ADAMTS).

DISCUSSION & CONCLUSIONS:We have developed a high reproducible 3D in vitro OA model characterized by quickly manufacturing, easy manipulation and large availability. This construct could be useful for huge applications such as testing new therapeutics, studies on cells crosstalk interactions.

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[3] DOI:10.1016/j.tvjl.2015.07.011
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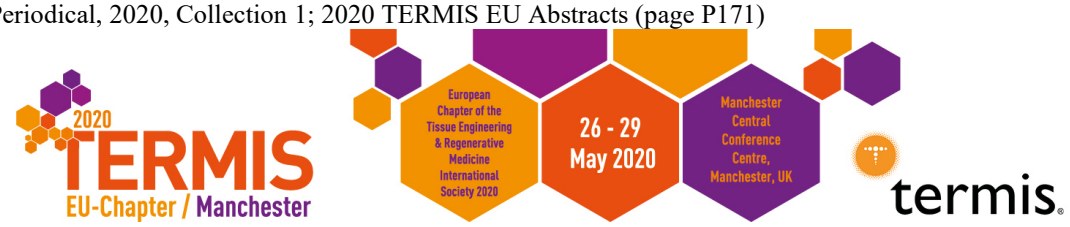
[5] DOI: 10.1016/j.stemcr.2015.05.005

Keywords: Disease models, Cartilage / joint and arthritic conditions



Developing tissue engineered models of the tumour microenvironment using self-assembling peptide hydrogels

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INTRODUCTION:The tumour microenvironment (TME) provides physical and chemical barriers preventing chemotherapeutic drug infiltration, leading to a poor clinical response. The TME is a diverse and complex interplay between many components, including extracellular matrix (ECM), vasculature and immune cells. However, many in vitro models used fail to recapitulate the TME and do not accurately represent the disease in question. Self-assembling peptide hydrogels (SAPH) are a class of biomaterials gaining popularity in tissue engineering, due to their synthetic design, biocompatibility and ability to host a variety of different cell types[1], [2]. This work aims to validate SAPH as a platform for in vitro modelling of cancer, as well as validating spatial profiling technology to assess gene expression of epithelial to mesenchymal transition (EMT) related markers in cell-laden hydrogels.

METHODS:MCF-7 and MDA-MB-231 breast adenocarcinoma cells were encapsulated within SAPH PeptiGelAlpha-1 (Manchester BIOGEL) for up to 14 days. Cell viability and proliferation were assessed throughout the culture duration. Immunohistochemical staining was used to observe ECM deposition and EMT related markers. Transmission electron microscopy (TEM) was carried out to observe cell-material interactions. Cell-laden gels were treated with doxorubicin and tamoxifen, followed by cell metabolic activity and proliferation assessment. Gene expression of EMT related markers was performed using qPCR and spatial profiling.

RESULTS:PeptiGelAlpha1 supports the viability and proliferation of MCF-7 and MDA-MB-231 cells, representing early stage and metastatic breast cancer respectively. Histological staining shows that MCF-7 cells arrange in tightly formed spheroids within the gel, whereas MDA-MB-231 cells prefer to remain dispersed. MCF-7 cells express mesenchymal markers such as N-cadherin and vimentin after 14 days, suggestive of EMT. MCF-7 cells remodel the hydrogel, via phagocytic uptake after 14 days. The number of hypoxic cells increases over 14 days. Diffusion of doxorubicin into SAPH was reduced when compared with tissue culture plastic, showing that the hydrogel provides a physical barrier to drug penetration, a more physiologically relevant setting than 2D cultures. Drug toxicity and gene expression data will also be presented.

DISCUSSION & CONCLUSIONS:In conclusion, our data support the use of SAPH for in vitro modelling of solid tumours and potentially to test anti-cancer drugs.

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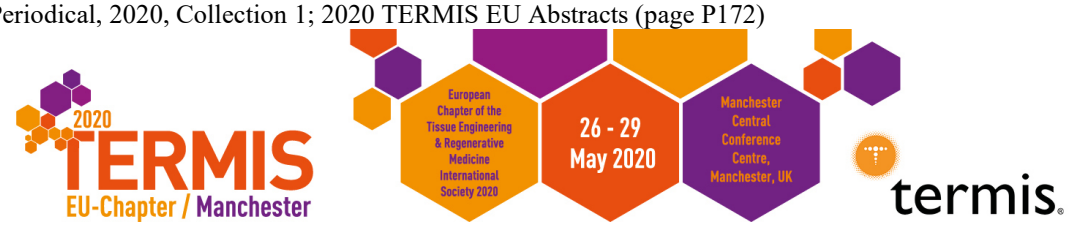
Keywords: In vitro microenvironments, Cancer



Using tissue engineered cartilage to investigate diabetes-induced osteoarthritis

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INTRODUCTION:People with diabetes have more than double the incidence of osteoarthritis.
Despite the huge and increasing global incidences of both diabetes and osteoarthritis, research on diabetes-induced osteoarthritis (DiOA) as a specific condition is very limited, partly because neither animal models nor traditional cell culture are capable of fully replicating the disease state. We have generated a lab-grown tissue engineered human model of diabetic osteoarthritis to investigate the effects of high glucose concentrations on chondrocytes, uncover the DiOA mechanism and determine the efficacy of several interventional treatments (including ascorbic acid and aspirin) to reduce cartilage damage in people living with diabetes and hyperglycemia.

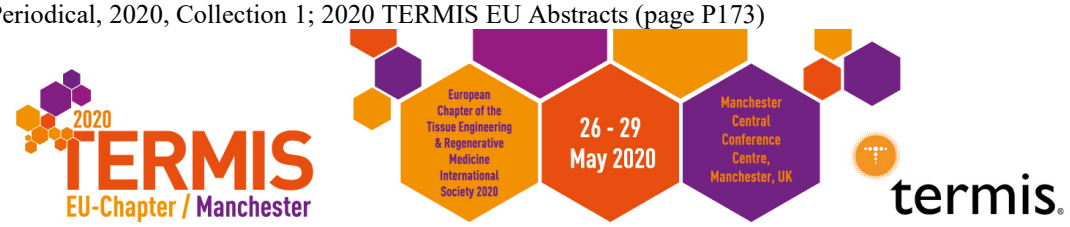
METHODS:Tissue engineered cartilage combines human chondrocytes with an agarose- or alginate-based hydrogel to support chondrocytes in a transparent matrix, recreating the actual cell density and tissue function of native adult cartilage.
The TE cartilage was cultured in either ‘normal’ 1g/l (5mM) glucose, high (4.5g/l; 25mM) or very high (10g/l; 55mM) glucose concentrations for 21 days. Media assays were performed to determine the synthesis and secretion of collagen, glycosaminoglycans and total protein by the chondrocytes. Interventional compounds were added to the media to test the targeted protective effects of ascorbic acid and aspirin (acetylsalicylic acid) on chondrocyte ECM production.

RESULTS:Chondrocytes survived in very high glucose concentrations (>10mg/ml) with little loss in viability, but their production of collagen and other proteins over 21 days was inhibited by high glucose. The production of glycosamino-glycans (sGAGs) was unaffected even in very high glucose-containing media. Supplementation with ascorbic acid (vitamin C), resveratrol and tocopherol (vitamin E) mitigated the effects of hyperglycemia, whilst high doses of acetylsalicylic acid (aspirin) were shown to completely reverse the disease phenotype and return collagen production to normal levels.

DISCUSSION & CONCLUSIONS:Diabetes-induced osteoarthritis may be a unique type of OA with a treatable cause. Our current research focuses on understanding the mechanism for the condition and evaluating dietary supplements that may mitigate or reverse cartilage damage caused by persistent hyperglycaemia. The role of mitochondria as key regulators of the disease is currently under investigation.

ACKNOWLEDGEMENTS:Financial support was received from the Dowager Countess Eleanor Peel Trust.

Keywords: Cartilage / joint and arthritic conditions, Disease models



Novel Matrices for Cancer and Bone Tissue Engineering Applications Based on Newly Generated Bioactive Enzymatically-Crosslinked Silk Fibroin Hydrogels

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INTRODUCTION:Silk biomaterials have shown promising biomedical applications in bone tissue engineering (TE), given its capacity to induce osteogenic signalling. In this study, we propose novel horseradish peroxidase (HRP)-enzymatically crosslinked silk fibroin (eSF) hydrogels, making use of CaO_2 as oxidizer, for finding applications as mineralized 3D bone-mimicking constructs and 3D in vitro tumor models of bone metastasis.

METHODS:This work proposes the development of novel HRP-crosslinked eSF hydrogels, by using SF solution at 8 wt.% concentration and calcium peroxide (CaO_2) as a substrate for the HRP, the so-called Ca-eSF. The obtained Ca-eSF hydrogels were compared to eSF hydrogels crosslinked with hydrogen peroxide (controls). The generated eSF hydrogels were structurally and physicochemically analysed. The in vitro response of eSF hydrogels was investigated by human osteosarcoma (SaOs-2) cells' encapsulation. The angiogenic potential of the hydrogels was tested by means of a CAM assay, and the in vivo biocompatibility was investigated by subcutaneous implantation in CD-1 and nude CD-1 mice for 3 and 6 weeks.

RESULTS:The generated Ca-eSF hydrogels undergo spontaneous protein conformational change from random coil to β-sheet during time. They showed hydroxyapatite-like crystals formation after 7 days of soaking in a simulated body fluid (SBF) solution. Cryo-SEM imaging revealed that these hydrogels possess a smaller porous internal microstructure, as compared to the control ones. In vitro, Ca-eSF hydrogels supported SaOs-2 cancer cells proliferation only in the amorphous hydrogel conformation, whereas the transition to β-sheet induced cells death. The CAM assay revealed that Ca-eSF hydrogels do not allow blood vessels infiltration. In addition, they induced surrounding tissue cells death after 3 and 6 weeks of subcutaneous implantation in mice.

DISCUSSION & CONCLUSIONS:In this study, novel HRP-crosslinked eSF hydrogels using CaO_2 as substrate (Ca-eSF) were developed. These Ca-eSF hydrogels showed a high in vitro bioactivity and a compacted porous microstructure, making them suitable matrices for bone TE. Preliminary data showed that the bioactive Ca-eSF hydrogels can inhibit angiogenesis and mediate in vitro and in vivo cells death after β-sheet conformational transition. Overall, the obtained results highlight the possibility of using these bioactive matrices for novel anti-tumor therapeutic strategies.

ACKNOWLEDGEMENTS:This work is supported by the projects FROnTHERA-RL1 (Ref. NORTE-01-0145-FEDER-000023), and by EU Framework Programme for Research and Innovation H2020 on FoReCaST under grant agreement no.668983. The FCT distinctions IF/01285/2015 and IF/00115/2015 under the Investigator FCT program are also greatly acknowledged.

Keywords: Hydrogels and injectable systems, Cancer

Adipose stromal/stem cells show protective functions on SH-SY5Y- and hiPSC-neurons after oxygen-glucose deprivation (OGD) induced damage in human in vitro stroke model

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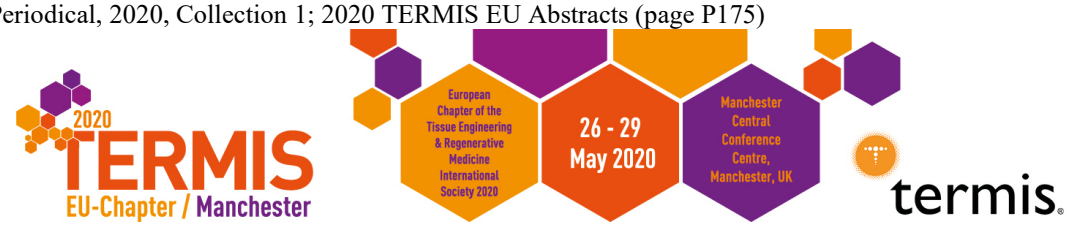
INTRODUCTION:Stroke is a devastating neurological disorder and one of the leading causes of mortality and disability. It is induced by compromised supply of blood to central nervous system leading to a deficiency of oxygen and glucose and consequently to neural injury. Knowledge of disease pathogenies is mostly focused on in vivo and in vitro studies in rodents, although numerous macrostructural, cellular and molecular discrepancies exist compared to humans. Human cell models would provide valuable information of disease mechanisms of action and reveal new therapeutic targets. Moreover, stem cell therapies are under investigation to treat patients after the stroke. To understand the cellular and molecular mechanisms of the stroke and the effect of stem cell therapy, an effective and repeatable in vitro human model needs to be developed. Aim of the study was to develop human cell based in vitro stroke model with oxygen-glucose deprivation (OGD) conditions and study the paracrine effects of human adipose stromal/stem cells (hASC) on neurons in co-culture system.

METHODS:In the present study, human neuroblastoma (SH-SY5Y) cell line and human induced pluripotent stem cells (hiPSC) were differentiated toward to neurons and then cultured under OGD conditions for 24 hours at 1% O2 in glucose-free medium. Thereafter, cells were transferred back to normoxic conditions with glucose for 0, 24 and 72 hours (reperfusion period) and co-cultured with hASCs. The effect of OGD and hASC was studied on the viability, apoptosis, proliferation and axonal damage of the neurons.

RESULTS:The results showed that OGD treatment induced cytotoxicity and apoptosis in SH-SY5Y and hiPSC-neurons. OGD-treated cells displayed less proliferation and their axonal networks were more damaged. The co-culture with hASCs showed protective function to neurons, since decreased number of dead cells and increased proliferation of both cell types were detected with hASC-treated neurons when compared to respective controls. Moreover, hASCs showed to protect SH-SY5Y cells from apoptosis after the OGD.

DISCUSSION & CONCLUSIONS:As a conclusion, we developed human in vitro stroke model with both human neuroblastoma cell line and hiPSC –derived neurons with oxygen-glucose deprivation (OGD) conditions. In this study, hASCs showed neuroprotective effect by increasing the cell proliferation after the OGD on neuronal differentiated SH-SY5Y cells and hiPSCs. In addition, ASCs decreased the apoptosis of SH-SY5Y cells, but similar effect was not seen in co-cultures with hiPSCs.

Keywords: Nervous system (brain-central-peripheral / disorders), Multipotent (mesenchymal) stem cells



Development and Evaluation of In Vitro Cardiac Hypertrophy Model by Mechanical Stimulation with Mir-21

Myeongjin SONG
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INTRODUCTION:Cardiac hypertrophy is accompanied with a number of heart diseases, including ischemic heart disease, hypertension, valve disease, and heart failure, which are associated with high morbidity and mortality. Cardiomyocytes in cardiac hypertrophy respond to hemodynamic stress, decrease cardiac beating and increase in their size, resulted in cardiac remodeling. However, underlying molecular and cellular mechanisms are not well known yet. Recent studies reported that injured cardiac fibroblast-derived mediators may act in autocrine and paracrine communications between fibroblasts and cardiomyocytes to induce cardiac remodeling.

METHODS:In this study, we developed multi-modal system mimicking hypertrophic conditions including hypoxia, mechanical loading and chemical agents. For mechanical stimulation, we used the magnetic particle coated with WGA (Wheat germ agglutinin) and exposed the controlled magnetic fields to apply the forces on the cell membrane.

RESULTS:To elucidate the effect of mechanical forces under the hypoxic condition cardiac fibroblasts and cardiomyocytes were cultured in the engineered hypertrophic condition, respectively. Cardiac fibroblasts were overexpressed the myo-firoblast marker such as ACTA2 (actin alpha 2, smooth muscle) and hypertrophic activator, microRNA-21 (mir-21) compared to the control cells. Cardiomyocytes showed the enlarged phenotype, overexpressed the cardiac hypertrophy markers, such as ANP (atrial natriuretic peptide), β-MHC (beta- myosin heavy chain), and CT-1 (cardiotrophin-1), and translocalized nucleus YAP (yes-associated protein) into the nucleus in combination with mechanical stimulus and mir21, indicating the hypertrophic characteristics.

DISCUSSION & CONCLUSIONS:These findings demonstrate that injured cardiac fibroblast secretes mir-21 as paracrine signaling mediators and affect cardiomyocytes, resulted in cardiomyocyte hypertrophy. We suggest that this cardiac hypertrophy system induced by mechanical stimulation and hypoxia is a promising alternative to an animal experimental model for studying cardiac disease, as well as a drug screening system for finding therapeutic targets.

ACKNOWLEDGEMENTS:This work was supported by grant (No. NRF-2016M3A9B6947892) from the National Research Foundation of Korea, Republic of Korea.

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Keywords: Disease models,

Angiogenic and anti-inflammatory properties of micro-fragmented fat tissue

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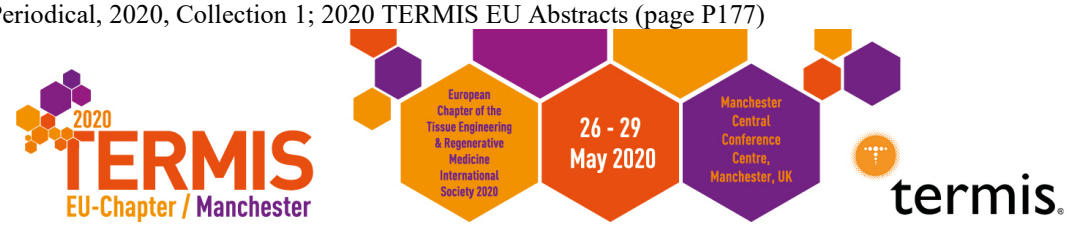
INTRODUCTION:Adipose-derived mesenchymal stromal cells (Ad-MSCs) are a promising tool for advanced cell therapy. In general, their isolation from fat lipoaspirate (LP) requires the use of enzymatic procedures, thus incurring in the complex requirements of GMP guidelines. Recently, to obtain Ad-MSCs through a minimal manipulation, an innovative device has been developed. This ready-to-use adipose tissue cell derivate – named Lipogems (LG), has been shown to have in vivo efficacy upon transplantation for ischemic and inflammatory diseases. In order to broaden our knowledge, we here investigated the angiogenic and anti-inflammatory properties of LG

METHODS:Human LG and their LG-MSCs were analyzed by immunohistochemistry for pericyte, endothelial and mesenchymal stromal cell markers. Angiogenesis was investigated by testing the conditioned media (CM) of LG (LG-CM) and LG-MSCs (LG-MSCs-CM) on cultured endothelial cells (HUVECs), evaluating proliferation, cord formation, and the expression of the adhesion molecules (AM) VCAM-1 and ICAM-1. The macrophage cell line U937 was used to evaluate CM anti-inflammatory properties, such as migration, adhesion on HUVECs, and release of RANTES and MCP-1.

RESULTS:We found that LG contained a very high number of mesenchymal cells expressing NG2 and CD146 (both pericyte markers) together with an abundant microvascular endothelial cell (mEC) population. Substantially, both LG-CM and LG-MSC-CM increased cord formation, inhibited endothelial AM expression ICAM-1 and VCAM-1 following TNFα stimulation, and slightly improved HUVEC proliferation. The addition of LG-CM and LG-MSC-CM strongly inhibited U937 migration upon stimulation with the chemokine MCP-1, reduced their adhesion on HUVECs and significantly suppressed the release of RANTES and MCP-1.

DISCUSSION & CONCLUSIONS:Our data indicate that LG is an effective preparation of micro-fragmented adipose tissue that retains either per se, or in its embedded MSCs content, the capacity to induce vascular stabilization and inhibits several macrophage functions involved in inflammation.

Keywords: Cell therapy, Immunity / immunomodulation / macrophage



3D Collagen scaffold organized with PLGA microcarriers for TGF-β1 controlled release: a study on hBM-MSCs chondrogenic commitment in static and dynamic cultivation

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INTRODUCTION:Treatment of cartilage lesions remains a major challenge owing to the avascular nature of the tissue, which prevents spontaneous healing. To date the use of Mesenchymal Stem Cells (MSC) and tissue engineering protocols represents an alternative strategy and offers great potential to improve joint cartilage therapy. The choice of 3D scaffold able to stimulate the formation of a new cartilaginous tissue is crucial to the success of the therapeutic strategy. Collagen-based scaffolds have been described as appropriate to support the chondrocyte attachment, because are more like natural ECM.

METHODS:A three-dimensional (3D) collagen scaffold was assembled as biomimetic extracellular matrix for human Bone Marrow Mesenchymal Stem Cells (hBM-MSC) induction towards chondrogenic phenotype by dispersing poly-lactic-co-glycolic acid (PLGA) microcarriers, carrying and delivering Transforming Growth Factor (hTGF-β1) payload, within the 3D structure. Cultivations were performed both in static than dynamic condition (perfusion).

RESULTS:hBM-MSCs early commitment towards a chondrogenic phenotype was confirmed both via immunofluorescence indicating Collagen type II fiber deposition within the scaffold and in the increase of gene expression of positive markers, such as Collagen2A1 and Sox9, after 16 days of cultivation. A better performance was monitored in dynamic cultivation.

DISCUSSION & CONCLUSIONS:The data presented provide evidence in support of the 3D described system present potential for use as a predictive in vitro model and as an implantable device supporting cartilage regeneration.

Acknowledgements:The authors acknowledge MiUR within the framework of PON-RI 2014/2020. Action I.1– "Innovative PhD with industrial characterization" Cycle XXXIV. PhD project: "Development of a 3D scaffold for local growth factor delivery to improve cartilage tissue engineering".

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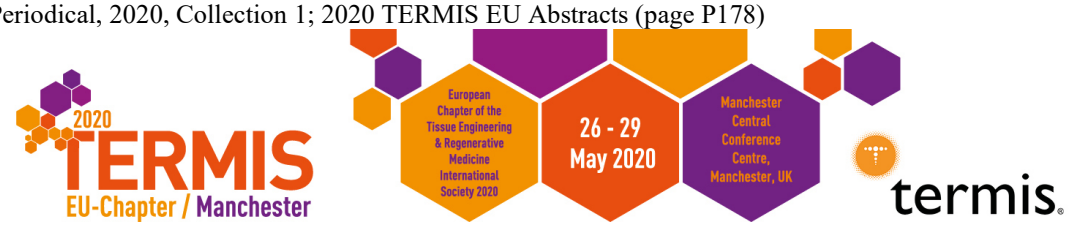
Keywords: Multipotent (mesenchymal) stem cells, Bioreactors



Targeting colorectal cancer: peptide modified CMCht/PAMAM dendrimer nanoparticles

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INTRODUCTION:Cancer cells from various sources have been reported to express high levels of the 67 kDa laminin receptor, the entity responsible for the interactions between cells and laminin of the extracellular matrix [1]. Specifically, the pentapeptide TyrIle-Gly-Ser-Arg (YIGSR), derived from laminin b1 chain, has been identified as the laminin binding site to its cell surface receptor. We hypothesized that attachment of YIGSR on the surface of previously developed CMCht/PAMAM dendrimer nanoparticles (NP) would lead to their preferential uptake by metastatic cancer cells over fibroblasts or endothelial cells.

METHODS:CMCht/PAMAM previously synthetized by our group were modified using simple EDC chemistry. After synthesis, nanoparticles were characterized using TEM. Sizes and surface changes of all nanoparticles were measured using zeta potential and DLS technologies.
The specific internalization of peptide-linked CMCht/PAMAM nanoparticles by colorectal cancer cells' was assessed in standard 2D culture flasks in a co-culture of live stained HCT-116 cancer cells (RED) and L929 fibroblasts (BLUE).

RESULTS:Herein, we describe a simple method to covalently link the YIGSR peptide to our dendrimer nanoparticles. Characterization techniques such as DLS show that the size of the modified nanoparticles increased when compared to the non-modified nanoparticles, with an increase from 54 to 130 nm (average). Also, the surface charge measured with Zeta Sizer were less negative compared to the unmodified NPs, indicating a reduction in the number of carboxylic acid groups on NP surface following peptide binding as well as the addition of the positively-charged arginine of the peptide sequence. Regarding NP's internalization, confocal studies after 24h of culture suggest in fact a targeted internalization by cancer cells, possibly overexpressing the laminin receptor. Quantitative studies as well as 3D studies are ongoing to confirm these promising findings.

DISCUSSION & CONCLUSIONS:The preliminary results confirm the successful modification of CMCht/PAMAM dendrimer nanoparticles with the peptide using simple EDC chemistry. Confocal images endorse the possibility of preferential internalization of peptide-modified NP in HCT-116 cancer cells. Moreover, we hope to improve the therapy efficiency for disseminated metastatic cancer without the currently drawbacks of low drug accumulation in target sites.

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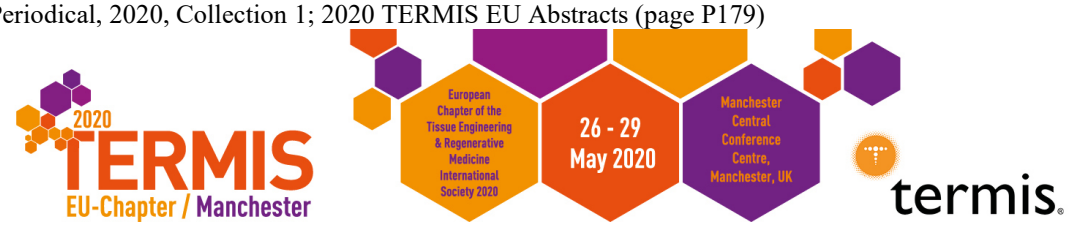
References:[1] Kumazoe M, J Clin Invest, 2013: 62:787-99.

Keywords: Drug delivery, Cancer



Targeted and controlled drug release by polymeric micelles sensitive to enzymatic- and redox-environment of arthritic diseases

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INTRODUCTION:Arthritic diseases are joint disorders associated with chronic synovial inflammation, pain and tissue damage [1]. As current treatments still present low efficacy and severe side effects, targeted and controlled drug release is an unmet need [2]. In this work, we propose polymeric micelles sensitive to the enzymatic- and redox-microenvironment present in the inflamed joints in order to increase the therapeutic index of dexamethasone (Dex) and reduce its side effects. After accumulation at the inflammatory site via the enhanced permeability and retention effect, the drug will undergo a quick release triggered by both redox and glutathione reductase (GR) activity.

METHODS:The linear copolymer composed of methoxypolyethylene glycol amine-glutathione-palmitic acid (mPEG-GSH-PA) was synthetized and glutathione (GSH) was oxidized intermolecularly to retain the drug. The size distribution, zeta potential as well as drug encapsulation efficacy and release from the polymeric micelles were all determined. Human articular chondrocytes, macrophages and endothelial cells were used to assess their cytocompatibility, and a co-culture model of articular inflammation was established to evaluate the beneficial role of encapsulating the drug into the micelles.

RESULTS:The developed polymeric micelles presented a uniform size of around 100 nm and a loading capacity of Dex of up to 65%. While in physiological conditions, the Dex release presented slow and sustained kinetics. In the presence of GSH and GR enzyme there is a dissociation of the disulfide links and consequently it is observed a burst release. After demonstrating their cytocompatibility below the concentration of 50 µg/mL, a co-culture system of chondrocytes and macrophages confirmed the beneficial role of encapsulating the drug into the micelles. Indeed, Dex encapsulated into the polymeric micelles, in the presence of GR and redox media, exhibited higher efficacy than the free drug. Importantly, as they were able to reduce the negative effects of Dex in normal cells, this strategy may provide important outcomes in arthritis treatment.

DISCUSSION & CONCLUSIONS:Our results demonstrate a targeted and controlled release of the drug from the micelles by the presence of redox medium and GR enzyme. Hence, this strategy will increase the drug therapeutic efficacy and reduce the severe side effects by limiting the exposure of healthy tissues to the drug. In conclusion, the developed polymeric micelles offer unique advantages for the treatment of arthritic diseases.

Acknowledgements:Financial support was received from FCT (PD/59/2013 and PTDC/CTM-BIO/4388/2014-SPARTAN) and NORTE 2020 Structured Project.

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Keywords: Nanomaterials (inc graphene), Cartilage / joint and arthritic conditions

Sweetening liposomes using a chemoenzymatic approach to target cancer cells

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INTRODUCTION:Lectins and carbohydrates on the cell surface are involved in key biological processes, such as cell-cell recognition. Since lectin-carbohydrate binding is usually weak, multiple links are required to achieve high affinity and specificity – the cluster glycoside effect.[1-3] Surface functionalization of liposomes, nanocarriers able to produce controlled and sustained release of drugs,[4] with saccharides is a promising strategy that will exploit the cluster glycoside effect to target specific cell types.[1]

This study aims to target cancer cells using liposomes functionalized with chemoenzymatically synthesized carbohydrates. By exploiting both the flexibility of chemical synthesis and the high regioselectivity/stereoselectivity of glycosyltransferases, complex oligosaccharides linked to functionalized spacer groups will be created.

METHODS:Two glycosyltransferases were used, namely β(1,4)-galactosyltransferase (β4Gal-T1) and T. cruzi trans-sialidase (TcTS). Cellular uptake of functionalized liposomes was evaluated using hepatocellular carcinoma cells (HepG2). Quantification of the conversion by β4Gal-T1 on vesicles was performed using liquid chromatography-mass spectrometry (LC-MS).

RESULTS:We have shown that both β4Gal-T1 and TcTS enzymes can be used in a 'one-pot' cascade to transfer galactose and N-acetylneuraminic acid, respectively, onto synthetic N-acetylglucolipids embedded in liposomes. Vesicles transformed with β4Gal-T1 showed the highest uptake by HepG2 cells.[5] Now the condensation of reducing sugars with hydrazide- and hydroxylamine-terminated lipids in a one-step reaction was used to increase the versatility of chemoenzymatic synthesis.[6] For example, a synthetic glycolipid was synthesised by attaching a hydroxylamine spacer onto cholesteryl chloroformate and subsequently reacting with N-acetylglucosamine (GlcNAc). After incorporation of the glycolipid into liposomes and incubation with β4Gal-T1, the addition of galactose was confirmed using vesicle agglomeration by Erythrina cristagalli lectin. Quantification by LC-MS showed a conversion in the range of 20-40%.

DISCUSSION & CONCLUSIONS:A high-throughput method has been developed for the immobilization of carbohydrates on liposome surfaces, using hydroxylamine lipids as the membrane anchors, and subsequent enzymatic transformation by β4Gal-T1. As future work, we plan to increase the complexity of the oligosaccharides on liposomes and evaluate the biological behaviour of these liposomes (containing doxorubicin) in cancer cells.

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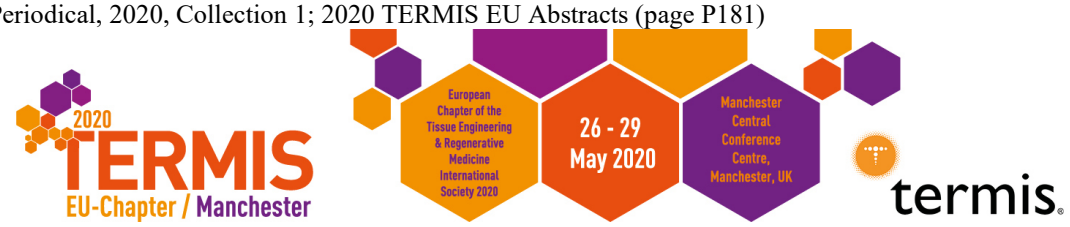
Keywords: Drug delivery, Cancer



An approach for vectorization of drug delivery from electrospun 3D matrices

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INTRODUCTION:Inhibition of neointima growth after percutaneous transluminal stenting with drug-eluted stents suggests the necessity to delivery of drug into artery wall, but not in bloodstream. To diminish getting of the drugs into blood from electrospun (ES) produced covering of vascular stents (VS) the inner layer filled with drug-adsorbing materials can be used. Here we study charcoal-based adsorbents for their capacity to bind/release drugs using anti-inflammatory drug diclofenac (DF) as an example.

METHODS:12 samples of Sibunit charcoal (S=22-275 m2/g), 7 types of carbon black (S=71-1505 m2/g) and 11 samples of active charcoal (S=650-2900 m2/g) were disaggregated in a ball mill up to 0.2-2 µm particles and characterized by SEM, nitrogen adsorption and DF binding capacity. The capacity of two most efficient DF binders – carbon black KetjenBlack 600 DJ (Lion Specialty Chemicals Co., Japan, 1520 m2/g) and active charcoal Anderson AX-21 (Anderson Development Company, USA, 2800 m2/g) to bind DF in blood was studied using tritium-labeled DF (3H-DF) [1]. To explore the possibility of adsorbents being introduced in ES produced fibers the conductivity of suspensions made from polycaprolactone solution in HFIP blended with KB-600 and AX-21 as well as films produced from the same suspensions were measured.

RESULTS:Isotherms of DF sodium salt adsorption are well linearized in Langmuir coordinates enabling to estimate equilibrium adsorption constants (Kads); the maximum adsorption was observed at equilibrium DF concentration of 80-200 mg/l. Kads vary in order of 3 for all tested DF adsorbents. Experiments with 3H-DF demonstrate similar character of DF adsorption in phosphate buffer (PBS), whereas in blood serum carbon black KB-600 selectively bound DF as compared to AX-21. Release of DF from adsorbents depends on adsorption conditions: long-term release was observed for DF bounded with KB-600 from PBS. Conductivity of KB-600 and AX-21 suspensions and films demonstrate that ES-produced fibers with 5-10% AX-21 can be obtained.

DISCUSSION & CONCLUSIONS:Charcoal-based materials are widely used as oral drugs and hemodialysis adsorbents and are not toxic and could be used for long term implantation as part of 3D matrices. Carbon black demonstrates good selectivity for DF in bio-liquids, promoting retention and vector delivery of DF from materials. Activated charcoal can be introduced in electrospun matrices and the properties of such materials planned to be studied.

Acknowledgements:The study was supported by RSF project 18-15-00080 and by RAS project AAAA-A17-117020210026-2.

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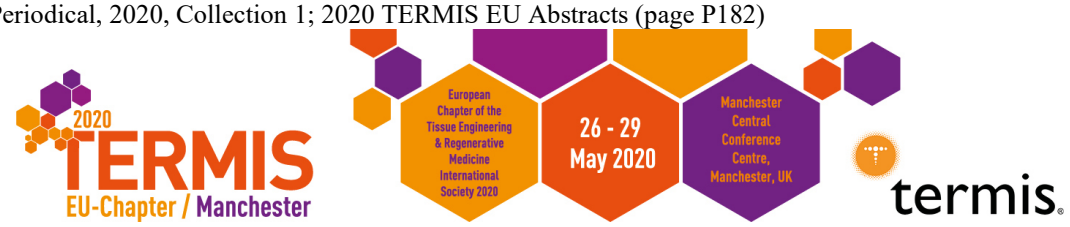
Keywords: Drug delivery, Biomaterials



Drug release from sirolimus enriched PCL-based matrices produced by electrospinning

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INTRODUCTION:To prevent neointima growth after angioplasty accompanied by metallic vascular stent (MVC) implantation, drug-eluting stents were offered [1]. Sirolimus (SRL) is among the most popular drug used for application onto such stents. Last studies demonstrate that SRL-eluting stents in a long time perspective demanded to be increased. Covering of MVC with fibrillary materials providing mechanical support of surrounding tissues and long-term drug release may increase their efficacy. Here we studied SRL enriched matrices produced by electrospinning (ES) and intended for coating of vascular stents.

METHODS:Tritium-labeled SRL (3H-SRL) was prepared by thermoactivated tritium exchange [2]. 150–180 μm matrices were obtained using ES on drum collector from the solution of polycaprolactone (PCL) with human serum albumin (HSA), dimethyl sulfoxide (DMSO), SRL and 3H-SRL. Matrices were characterized as previously described [3] and SRL release in static or dynamic conditions was evaluated by radioactivity of supernatants after incubation of matrices during different time intervals (up to 27 days) in PBS or in human blood plasma (HBP).

RESULTS:Preparations of 3H-SRL was obtained with radioactivity of ~0.055 Ci/mmol. 3H-SRL was combined with unlabeled SRL to reach a dose of ~0.9 μg/cm2 of SRL and radioactivity ~25,000 cpm/cm2. SRL release in PBS was close to previously published data for paclitaxel [3], suggesting diffusion dependent release. Introduction of HSA in ES solution increased the SRL release both in PBS and in HBP. Incubation of the matrices with HBP increases efficacy of SRL release obviously by binding of the drugs with serum biomolecules. Exchange of PBS or serum after every time point increases SRL release; in HBP SRL was released totally in 3-9 days. It was shown that matrices produced from ES solution with DMSO and HSA released no more than 75% of SRL after 27 days in BP even under medium exchange conditions.

DISCUSSION & CONCLUSIONS:The data obtained demonstrate the necessity to consider medium exchange in place of stent implantation. The use of plasma as the external medium accelerates SRL release. PCL-based 3D matrices containing HSA, SRL and DMSO can be used to produce vascular stents coatings providing prolonged SRL delivery.

Acknowledgements:The study was supported by a grant from the Russian Science Foundation no. 18-15-00080.

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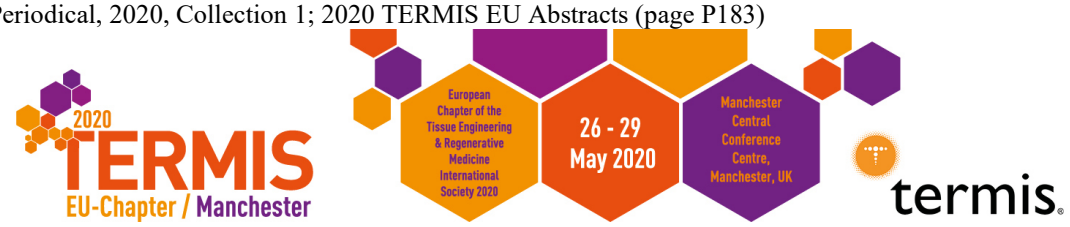
Keywords: Drug delivery, Vascular systems / vascularisation and heart



Enhanced biotherapeutic delivery through membrane engineered red blood cells

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INTRODUCTION:Biotherapeutics have achieved global success due to their high specificity towards their drug targets, providing exceptional safety and efficiency. The ongoing shift away from small molecule drugs towards biotherapeutics has heightened the need to further improve these biologics and their pharmacokinetics. The three ubiquitous obstacles that limit therapeutic capacity are proteolytic degradation, short half-life, and the development of anti-drug antibodies. One possible solution to limited pharmacokinetics is employing improved drug delivery systems, specifically coupling biotherapeutics to long-lived and biocompatible red blood cells (RBCs). Herein, we have shown the rapid and tunable chemical engineering of RBC membranes in order to display any therapeutic antibody by immobilizing anti-Tumor Necrosis Factor (anti-TNFα) on the surface of RBCs. Furthermore, we have recently initiated studies in the engineering of human butyrylcholinesterase (HuBChE) to the RBC membrane surface to serve as an enhanced prophylactic treatment against organophosphate nerve agents.

METHODS:The RBC-based antibody delivery system consisted of Staphylococcal protein A (SpA) conjugated to the RBC membrane surface via poly(ethylene glycol) (PEG). Following RBC-PEG-SpA synthesis, anti-TNFα antibodies were immobilized to the RBC surface via SpA-Fc binding. An ELISA assay and confocal imaging were used to observe antigen scavenging by the RBC-antibody constructs. The long-term stability of the constructs in the presence of human serum was quantified. An ektacytometer was implemented to measure membrane deformability of surface engineered RBCs. Identical conjugation chemistry was exploited to bridge HuBChE to the RBC membrane via a single PEG chain. Detailed kinetic studies were used to assess HuBChE activity of the RBC-HuBChE constructs.

RESULTS:The RBC-antibody constructs were shown to successfully scavenge antigen in solution. These constructs were shown to be stable for more than 6 weeks in the presence of human serum without the detection of antibody dissociation. The RBC constructs also displayed similar mechanical properties to unmodified RBCs. Furthermore, the RBC-HuBChE constructs demonstrated that HuBChE retains its activity after being immobilized onto RBCs.

DISCUSSION & CONCLUSIONS:We have designed, synthesized, and tested RBC-based platforms for carrying antibody therapeutics as well as nerve agent scavenging HuBChE. These results overall provide strong evidence for the ability of RBCs to serve as an enhanced biotherapeutic delivery platform for an array of biologics with intravascular drug targets. In future work, pharmacokinetic studies of these RBC-based drug delivery systems will be initiated in animal models.

ACKNOWLEDGEMENTS:This work was funded by the Institute of Transfusion Medicine and the Defense Threat Reduction Agency.

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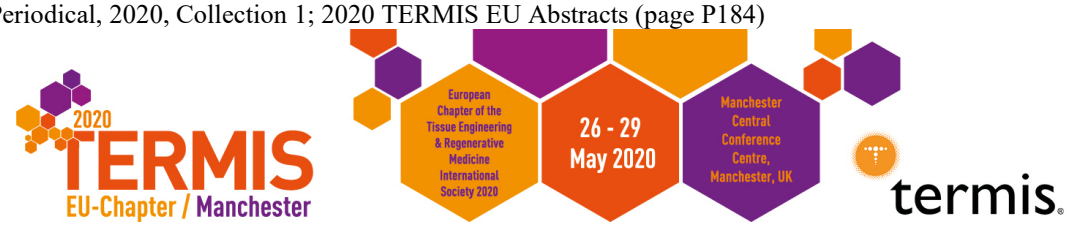
Keywords: Biologics and growth factors, Biomaterials



Design of stretchable drug delivery systems combining polyrotaxane-based hydrogels and lipid nanoparticles

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INTRODUCTION:A hydrogel acting as a stretchable vessel for the sustainable delivery of thymol, a natural active presenting anti-inflammatory properties, could serve for reducing joint inflammation and associated pain. Like many hydrophobic drugs, thymol cannot be loaded as such in hydrogels. Formulated in aqueous environment as lipid nanoparticles that could be stably embedded in semi-solid formulations, its transdermal administration could be facilitated.[1] Hydrogels are biomaterials of choice for the design of transdermal patches. To improve material mechanical properties, and in particular stretchability,[2,3] an original approach was investigated based on the use of polyrotaxanes (PR) as cross-linkers. Since their discovery in the 1990's by Harada and co-workers,[4] PR have attracted great interest for the design of smart materials with dynamic functionality, thanks to the supramolecular mobility of their cyclic compounds (rotation and sliding).[5] We therefore combined PR-based hydrogels and thymol-LNP for the design of innovative stretchable materials with anti-inflammatory sustainable release.

METHODS:The physical and chemical properties of thymol-LNP were characterized over time by DLS and HPLC studies. In a one-pot reaction, carboxylated β-cyclodextrins β-CD-(COOH)n were threaded on a thiol-modified poloxamer (PPG9-PEG24-PPG9) and end-capped with bulky alkene-functionalized-β-cyclodextrins through thiol-ene chemistry. The water-soluble PR obtained were thoroughly characterized thanks to 1H and 13C NMR experiments, FT-ICR Mass Spectrometry, TEM and AFM. The morphological and mechanical properties of PR modified with methacrylates groups/LNP hydrogels were characterized by SEM and rheometry.

RESULTS:Thymol-loaded LNP were obtained with an average diameter of 150 nm and presented over 6 months stability. PR with a controlled number of threaded cyclodextrins (2, 4, or 8 /PR) were synthetized and characterized. Dynamic rheological measurements demonstrated hydrogel formation by photopolymerization combining methacrylate-modified PR and LNP.

DISCUSSION & CONCLUSIONS:Thymol-loaded LNP were obtained with an average diameter of 150 nm and presented over 6 months stability. PR with a controlled number of threaded cyclodextrins (2, 4, or 8 /PR) were synthetized and characterized. Dynamic rheological measurements demonstrated hydrogel formation by photopolymerization combining methacrylate-modified PR and LNP.

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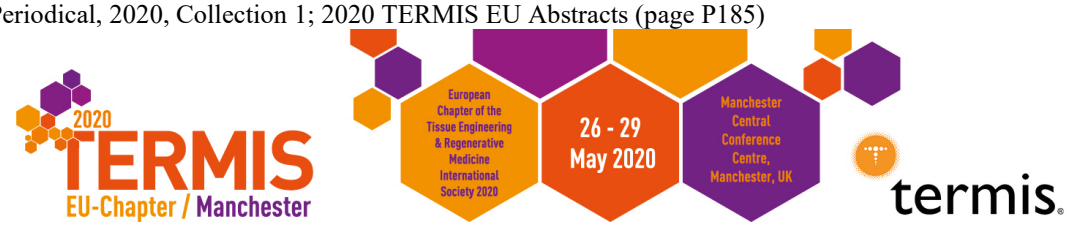
Keywords: Hydrogels and injectable systems, Nanomaterials (inc graphene)



Thematic Analysis of the Twitter Mentions in Tissue Engineering: an Altmetric-Based Analysis

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INTRODUCTION:Tissue Engineering (TE) engendered a therapeutic revolution in 20th-century science. A previous study analyzing the scientific production of TE in the Web of Science (WoS) using Science Mapping Analysis (SMA) demonstrated the increasing important of this field in the international literature (1). With social media, new actors have emerged (e.g., practitioners, undergraduate students, or lecturers), which read and perform critical analysis of research articles, and now also, share them. Thus, the main aim of this study is to analyze the most relevant themes mentioned on Twitter, one of the major tools to spread knowledge in our days.

METHODS:A set of documents were obtained from the WoS using the following query: ("TISSUE ENGINEER*" OR "TISSUE-ENGINEER*"). The list of DOIs of the articles and reviews published during the period 2012-2016 were retrieved to match them with the data of Altmetric.com. A total of 4,679 documents with at least 1 Twitter mention were analyzed. The SciMAT software (2) was used to perform the SMA and discover the most relevant themes.

RESULTS:Strategic diagram for the Twitter mentions of the Tissue Engineering production in the period 2012-2016 revealed that mesenchymal stem cells, hydrogels, angiogenesis, nanomaterials, and reparative medicine were motor themes as the showed high centrality and density. The first three of these were also motor themes according to WoS results.

DISCUSSION & CONCLUSIONS:According to the previous findings, mesenchymal stem cells, hydrogels, and angiogenesis are the motor themes in both WoS and Twitter platforms. In that way, there are relevant themes in WoS, but they lose importance on Twitter. The type of study and document are influencing the number of Twitter mentions, remarking the differences in actors' interest.

ACKNOWLEDGEMENTS:Supported by CTS-115, Tissue Engineering Group of the Department of Histology of the University of Granada, Spain.

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Keywords: Education, Other

Piezoelectric biomaterials for neural tissue engineering applications

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INTRODUCTION:Due to their electrical activity, nervous system reacts naturally to electrical signals. Tissue engineering research relies on the fact that when an injury takes place it is essential to re-establish and to mimic the specific tissue microenvironment. Taking advantage of electroactive piezoelectric materials for scaffolds development, electrical signals can be recreated making them a promising approach for neural repair. The surface charge of a material influences cellular adhesion, proliferation and differentiation, in particular when this surface charge can be dynamically varied, such as in the case of piezoelectric materials [1]. Surface charged piezoelectric poly (vinylidene fluoride) (PVDF) has proven to enhance material biofunctionality and has already been successfully used for bone and muscle TE [2]. This work reports on the evaluation on how surface charged PVDF films influence a neural-derived cell line.

METHODS:PVDF piezoelectric films are produced by solvent casting. Sample poling was achieved by Corona discharge inside a home-made chamber, obtaining poled samples (d33 ≈ 24 pC.N-1) with overall negative or positive surface charge.
The influence of electrically charged (positive and negative poled) PVDF films on SH-SY5Y cells were assessed through the viability assay MTS (3-(4,5-dimetiltiazol-2-il)-5-(3-carboximetoxifenil)-2-(4-sulfofenil)-2H-tetrazolium) and immunofluorescence assay, where the nuclei was labeled with 4',6-Diamidino-2-Phenylindole (DAPI) and the cytoskeleton integrity with phalloidin (FITC). Differentiation assays were also carried out.

RESULTS:The results shown that neural cells viability is significatively enhanced by negatively poled films, when compared to positively poled PVDF, after 48 and 72 h of cell culture. After the cell viability assays, the negative poled samples were used for the differentiation assays, demonstrating that the negative surface charge enhanced the neurite differentiation.

DISCUSSION & CONCLUSIONS:Negatively poled PVDF films demonstrated enhanced neuron cellular viability, when compared to positively poled PVDF films. It is shown that surface electrical polarization influenced neuronal cellular adhesion and proliferation, demonstrating its promising application for dynamic studies where the surface charge can be modulated through mechanical stimulus.

ACKNOWLEDGEMENTS:Acknowledgements:Fundação para a Ciência e Tecnologia: UID/FIS/04650/2019, UID/BIA/04050/2019, UID/EEA/04436/2019 and UID/QUI/0686/2019, projects PTDC/EMD-EMD/28159/2017, PTDC/BTM-MAT/28237/2017 and grant SFRH/BD/141136/2018 (TMA). Spanish Ministry of Economy and Competitiveness (MINECO): MAT2016-76039-C4-3-R. Basque Government: ELKARTEK, HAZITEK, and PIBA (PIBA-2018-06) programs.

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Keywords: Biomaterials, In vitro microenvironments

Electroactive Ionic Liquids based polymer blends: a new approach for active tissue engineering

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INTRODUCTION:Ionic liquids (ILs) are a diversified group of salts composed of organic cations and a variety of anions that present a melting point below 100°C. Their properties such as negligible vapor pressure, high ionic conductivity, electrochemical and thermal stability, as well as the ability to be used as green solvents, make them suitable for the development of smart materials with new/or improved functionalities and applications [1]. Combining ILs with a polymeric matrix offers advantages when compared to conventional materials, allowing to combine the key features of polymers and the ILs characteristics. Those ionic liquid based smart materials are gaining increasing attention in several areas, with a strong potentiality in tissue engineering (TE) [2]. This work focuses on advanced materials and strategies concerning ILs combined poly(vinilidene fluoride) (PVDF) for TE applications.

METHODS:Biocompatible ionic electroactive materials based on PVDF and ILs 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]) and 2-hydroxyethyl-trimethylammonium dihydrogen phosphate ([Ch][DHP]) were developed comprising different IL contents (10, 20 and 40% wt.). The morphological, physico-chemical and thermal properties of the composites were analysed, as well as their potential as scaffolds for muscle TE was evaluated by cytotoxicity and cell proliferation assays.

RESULTS:Results demonstrated that the [Bmim][Cl] and [Ch][DHP] incorporation into the PVDF matrix influences the microstructure and also PVDF crystallization process. An increase in PVDF electroactive β-phase content and crystallinity is obtained with the presence of both ILs, indicating that they act as nucleating agent for polymer crystallization process. Besides of enhancing the electrical conductivity, the ILs incorporation also influences the thermal properties of the films. Regardless of the IL used, it is observed a decrease in the Young´s modulus, indicating that ILs act as a PVDF plasticizer whitin the polymeric matrix. Finally, cell culture assays showed that cells adhered and proliferated in all samples. The myoblast cells are elongated and present a compact cytoskeleton particularly when growing on [Bmim][Cl]/PVDF.

DISCUSSION & CONCLUSIONS:The noncytotoxicity of the IL/PVDF composite films and C2C12 cell proliferation demonstrate the applicability of the developed materials as a suitable platform for active muscle regeneration strategies.

Acknowledgements:Portuguese Foundation for Science and Technology (FCT):UID/FIS/04650/2019, UID/BIA/04050/2013, UID/BIO/04469/2019, projects PTDC/EMD-EMD/28159/2017, PTDC/BTM-MAT/28237/2017 and grants SFRH/BD/148655/2019 (R.M.M.), SFRH/BPD/121526/2016 (D.M.C.) and SFRH/BD/111478/2015 (S.R.) grants. Spanish Ministry of Economy and Competitiveness (MINECO):MAT2016-76039-C4-3-R (AEI/FEDER, UE). Basque Government: ELKARTEK, HAZITEK, and PIBA (PIBA-2018-06).

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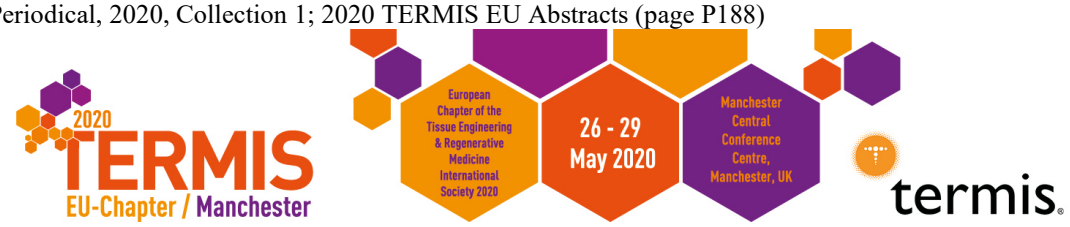
Keywords: Biomaterials, Electroactive materials



A novel in vitro system for engineering the development of human tissues using human pluripotent stem cells

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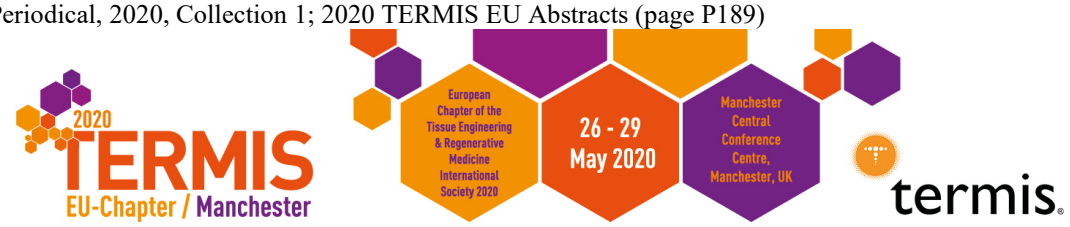
INTRODUCTION:Since human embryonic stem cells (hESCs) were first isolated in 1998 and later shown to propagate as individual lines in vitro, they have caught the attention from the scientific community due to their potential use as a raw material for the production of therapeutically useful cell types such as pancreatic β cells, cardiomyocytes and motor neurons. Similar to hESCs, human induced pluripotent stem cells (hiPSCs) have the capacity to differentiate into several cell types and can be useful as a tool for disease modelling and drug screening. Any new hESC or hiPSC line, must be characterized and confirmed its pluripotency before proceeding with downstream applications; particularly, if the cell line is be used for clinical applications. To test their pluripotency, researchers use the teratoma assay which involves the injection of undifferentiated pluripotent stem cell line into immunodeficient mice leads usually to growth benign tumours called teratomas. Despite being the gold standard assay, there is little consistency in either the methodology used or the reporting of results.

METHODS:We used a novel strategy that combines the culture of size controlled of human embryoid bodies (hEBs) in suspension followed by their prolonged maintenance and differentiation in a 3-dimensional porous scaffold (Alvetex, Reprocell) that enables cells to form complex and organized structures. Histological staining and immunofluorescence for specific markers revealed the identity and complexity of the tissues formed.

RESULTS:We observed that initial number of cells and size of the embryoid body directed the cell fate without the addition of external factors. On the other hand, the model improved cell viability, extending their time in culture and enhancing the formation of complex 3D tissues. Using exogenous factors such as BMP4, Activin A, retinoic acid, we increased the complexity of the structures and we were able to direct the differentation towards a specific lineage.

DISCUSSION & CONCLUSIONS:This model has the potential to be used as a novel, animal free alternative to assess the pluripotency of human pluripotent stem cells. At the same time, our findings may help to understand the factors that affect hESC and hiPSC differentiation such as morphogen and growth factors, size of hEBS and interaction with other cells.

Keywords: Differentiation, Stem cells – general



Extracellular Vesicles (EVs) Released in the Presence or Absence of Inflammatory Cues from Human Cardiac-derived Cells Support Angiogenesis in Different Manners

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INTRODUCTION:Extracellular vesicles (EVs) from regenerative cells are considered as a new therapeutic approach to treat diverse diseases like cardiovascular diseases. Since the cargo of EVs is crucially influenced by the microenvironment during their biogenesis, it is of major interest to evaluate what might be the most suitable condition for a latter manufacturing phase. To elucidate the effect of pro-inflammatory cytokines, we generated EVs from the conditioned medium derived either in the presence or absence of a pro-inflammatory cytokine cocktail (IFNγ, TNFα and IL-1β) from human cardiac-derived adherent proliferating (CardAP) cells. CardAP cells were chosen, because their conditioned medium was recently shown to enhance angiogenesis [1].

METHODS:In this study, CardAP-EVs were isolated by differential ultracentrifugation and afterwards evaluated for their cargo of miRNAs as well as their effects on endothelial cells (release of soluble factors, tube formation, up-take capacity).

RESULTS:We were able to show that murine recipient cells internalized CardAP-EVs as determined by an intracellular detection of human proteins, such as CD63, by a novel flow cytometry method for studying EV–cell interaction. Moreover, endothelial cells exhibited a higher tube formation capability on Matrigel when either treated 24 h prior with unstimulated or cytokine stimulated CardAP-EVs. Interestingly, unstimulated CardAP EVs caused endothelial cells to release significantly more vascular endothelial growth factor (VEGF) and interleukin (IL)-6, while cytokine stimulated CardAP-EVs significantly enhanced the release of IL 6 and IL-8. By nCounter® miRNA expression assay (NanoString Technologies) we identified microRNA 302d-3p to be significantly enhanced in unstimulated CardAP EVs compared to their cytokine stimulated counterparts, which was verified by quantitative polymerase chain reaction. MicroRNA 302d-3p was shown in a previous study to cause increased levels of VEGF and tube formation capabilities [2].

DISCUSSION & CONCLUSIONS:In conclusion, unstimulated and cytokine stimulated CardAP-EVs are pro-angiogenic by inducing different factors from endothelial cells respectively. It allows us to select in future potent targets for a safe and efficient therapeutic application for treating cardiovascular diseases.

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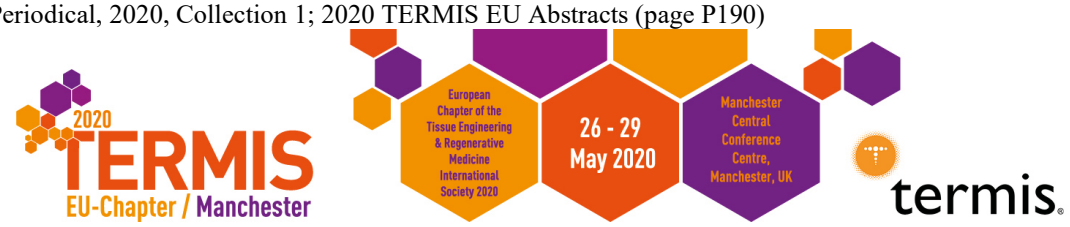
Keywords: Cell therapy, Vascular systems / vascularisation and heart



Combining extracellular vesicles with porous electrospun scaffolds for cell-free tissue engineering

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INTRODUCTION:Tissue and organ failure or damage represents a significant healthcare problem. Tissue engineering provides potential solutions to solve this problem. Extracellular vesicles (EV) are membrane-enclosed vesicles secreted by various cell types and include exosomes, microvesicles and others1. EVs play an important role in cell- cell communication and tissue repair and therefore offer opportunities for novel tissue engineering approaches. Here, we aimed to incorporate EV into porous electrospun scaffolds to be used for different tissue engineering applications.

METHODS:EV were isolated from the conditioned media of cells in culture using ultracentrifugation (UC) and size exclusion chromatography (SEC). EV properties were examined using ZetaView and q-Nano. Polycaprolactone (PCL) scaffolds were fabricated by electrospinning and characterized by SEM and TEM. After adding EVs to the scaffold, EV incorporation was characterized by SEM, TEM and fluorescent microscopy.

RESULTS:EV were successfully isolated by UC and SEC as assessed by particle tracking analysis (Zetaview) and determination of presence of EV marker proteins (CD9, CD63 and CD81). EVs were observed as spherical and cup shaped under TEM and SEM, respectively. EV tended to aggregate when isolated by UC in comparison to SEC. The size of fibres of PCL electrospun scaffold ranged between (600 nm- 5 µm) with average diameter (1.7 µm ± 0.82µm). Successful incorporation of EVs within PCL scaffolds was observed by SEM, TEM and fluorescent microscopy.

DISCUSSION & CONCLUSIONS:The aggregation of EVs isolated by UC was observed in this study, which may affect the distribution of EVs within the scaffold.
We observed that isolation by SEC prevented aggregation. Here we provide evidence that EV can be incorporated in electrospun scaffolds, offering an opportunity to develop novel tuneable, cell-free approaches to tissue engineering.

ACKNOWLEDGEMENTS:The NanoView biosciences is thanked for providing access to Exoview for characterising EV tetraspanin markers. Also to Mr. Christopher J Hill for helping with SEM and Areeg A Elmusrati for helping with SEC protocol.

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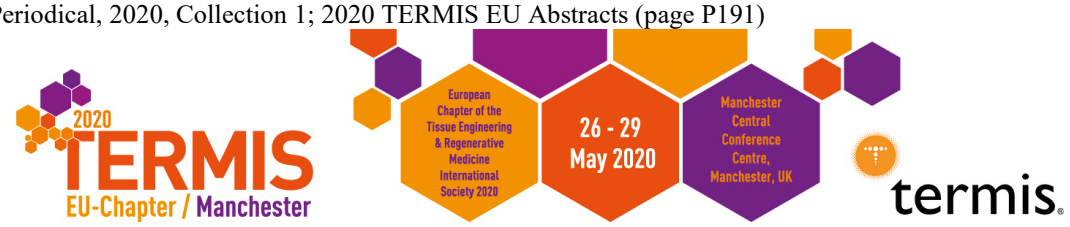
Keywords: Extracellular vesicles, Biomaterials



The therapeutic potential of extracellular vesicles in preclinical stroke models: a systematic review and meta-analysis

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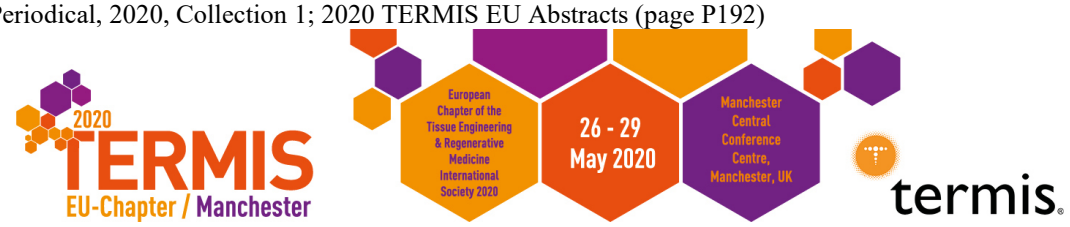
INTRODUCTION:Stroke is a global health problem, responsible for 6.7 million deaths annually, and resulting in disabilities in a third of survivors. Currently there is a paucity of clinically available regenerative therapies for stroke. Extracellular vesicles (EVs) are nanoscale membrane-bound vesicles which carry cargoes including DNA, RNA and proteins that are involved in intercellular signalling. EVs have recently been investigated for their potential as modulators of regeneration in the post-stroke brain; this systematic review and meta-analysis aims to provide a summary of the efficacy of therapeutic EVs in preclinical stroke models, to inform future research in this emerging field.

METHODS:Studies were identified by a comprehensive literature search of two online sources and subsequent screening. Studies utilising lesion volume or neurological score as outcome measures were included. Standardised mean difference (SMD) and 95% confidence intervals were calculated using a restricted maximum-likelihood random effects model. Publication bias was assessed with Egger's regression and presented as funnel plots with trim and fill analysis. Subgroup analysis was performed to assess effects of different study variables. Study quality and risk of bias were assessed using the CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data in Experimental Studies) checklist.

RESULTS:A total of 20 publications were included in the systematic review, of which 19 were assessed in the meta-analysis (43 comparisons). Overall, EV interventions improved lesion volume (SMD: -1.95, 95% CI: -2.72, -1.18) and neurological scores (SMD:-1.26, 95% CI: -1.64, -0.87) compared to control groups. Funnel plots were asymmetrical suggesting publication bias, and trim and fill analysis predicted 7 missing studies for lesion volume. Subgroup analysis suggested administration at 0-23 hours post-stroke was the most effective timepoint for EV treatment. The median score on the CAMARADES checklist was 7 (IQR: 5-8).

DISCUSSION & CONCLUSIONS:EVs may offer a promising new avenue for stroke therapies, as EV-based interventions had positive impacts on lesion volume and neurological score in preclinical stroke models. Subgroup analyses may have been confounded by considerable heterogeneity in study design and the low number of included studies. However, given their efficacy across studies with notably different treatment regimens, the positive effects of EVs may be wide ranging in the contexts of neuroprotection, repair and regeneration. Interest in therapeutic EVs is expanding, with study numbers increasing yearly, therefore we expect that a clearer understanding of the most effective EVs and treatment regimens may soon come to light.

Keywords: Cell therapy, Nervous system (brain-central-peripheral / disorders)



Pro-Angiogenic Secretome Derived From Mesenchymal Stem Cells. A Potential Strategy Promoting Tissue Biointegration in Tissue Engineering

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INTRODUCTION:Human mesenchymal stem cells (MSC) may have important regenerative effects in tissue engineering. However, previous reports suggest that their effect may be mediated by the release of a complex secretome (1,2). In this work, we have analyzed the potential of the secretome of several types of human MSC to stimulate angiogenesis, proliferation and cell survival as a strategy for graft regeneration in skin and oral mucosa.

METHODS:Wharton jelly (HWJSC), dental pulp (DPSC) and bone marrow stem cells (BMSC) were isolated and cultured. Subsequently, the secretome of each cell type was isolated. In order to determine the angiogenic potential of each secretome, a proteomic study was carried out against 55 proteins involved in the process of blood vessel development (Proteome ProfilerTM Array). Additionally, the effects of the secretome on cell survival and cell proliferation was analyzed by WST-1 and LIVE/DEAD studies. For these, human skin fibroblasts were incubated with secretome isolated from HWJSC, DPSC and BMSC for periods of 24 and 72 hours. Finally, statistical significance of the results obtained was determined by a Mann-Whitney U statistical test.

RESULTS:The proteomic analysis of secretome isolated from each MSC revealed the presence of 10 proteins involved in the angiogenesis process, including IL-8, MCP-1, PTX3, Serpin E1, Serpin F1, TIMP-1, TSP-1, uPA, ANG and IGFBP-3. In addition, the analysis of WST-1 study revealed significant differences in terms of proliferation between HWJSC and DPSC secretomes at 24 and 72h. Additionally, cell viability as determined by LIVE/DEAD assay of human skin fibroblasts cultured with secretome showed a significant cell viability values, especially in the BMSC group.

DISCUSSION & CONCLUSIONS:The use of the secretome obtained from mesenchymal stem cells may be a potential strategy contributing to increase the natural process of tissue biointegration mediated by neovascularization in the host tissue. We therefore conclude that secretomes obtained from MSC could be incorporated to the biomaterials of tissues generated by tissue engineering before in vivo grafting, especially in the case of tissues requiring rapid neovascularization such as the human skin and oral mucosa.

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Keywords: Extracellular vesicles, Multipotent (mesenchymal) stem cells

Succinyl cholesterol grafted to hyaluronan improves formulation to treat dry eye syndrome

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INTRODUCTION:The global prevalence of dry eye syndrome is estimated up to 50% worldwide. In the diseased condition, essentially functional tear films are no longer produced physiologically in the eye. The immediate and least invasive treatment for dry eye syndrome is the use of artificial tears, which are generally based on mucoadhesive polymers that protect the ocular surface. Preclinical and clinical studies have shown that artificial tears containing hyaluronan (HA), provide acute and long-term therapeutic benefits. Unfortunately, HA has the limitation of rapid clearance and limited adhesion; therefore, the commercially available products need frequent re-instillation.

METHODS:In this work, we would like to discuss the development of a novel formulation based on an amphiphilic hyaluronan, which consist of succinyl cholesterol grafted to HA (HA-SCH). The esterification reaction of HA was mediated by mixed anhydrides(Huerta et al, 2018). The chemical characterization of HA-SCH was elucidated using NMR spectroscopy, SEC-MALLS, and GC. The aggregation properties of the HA- SCH derivatives was studied by DOSY NMR, and encapsulation of Nile Red emission wavelength shift. A 2D dry eye model was developed using human keratinocytes (HaCaT) was developed to evaluate the effectivity of the novel composition versus over-the counter products (obtained in the EU market). A rheological characterization of the novel composition was performed by a Malvern Kinexus pro+ rheometer.

RESULTS:A perfect control of the degree of substitution was obtained by varying the molar ratio of cholesterol used in the reaction feed. Also, the effect of Mw of starting hyaluronan was evaluated towards control of the viscosity. A low cac value was obtained for HA-SCH. Moreover, the derivative was able to encapsulate 10 % wt. of Coenzyme Q10, which has been potentially used for treating patients with mild to moderate dry eye (Postorino et al., 2018). The developed eye drops formulation containing HA-SCH was more effective than commercially available products as well as parent HA

DISCUSSION & CONCLUSIONS:We have developed a precise physicochemical characterization based on amphiphilic HA. The model can predict the in vitro efficacy. Thus, amphiphilic HA derivatives represents a new possibility in dry eye treatment.

Acknowledgements:This research was supported by the European Regional Development Fund - Project INBIO (No. CZ.02.1.01/0.0/0.0/16_026/0008451).

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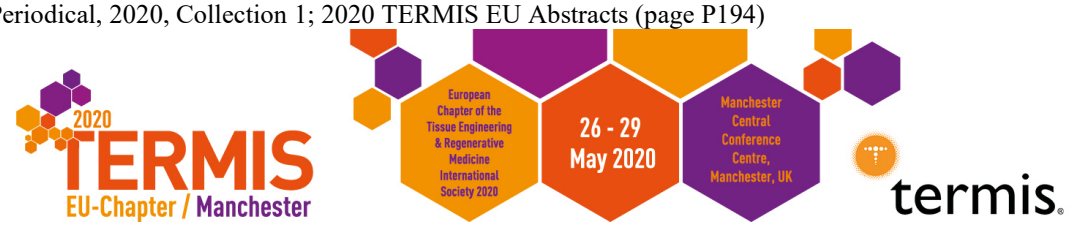
Keywords: Polymers - natural / synthetic / responsive, Drug delivery



Low-temperature vacuum-evaporation as a devitalisation and preservation technique for long-term storage and ambient distribution of transplantable human corneas

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INTRODUCTION:A review from 2016 conservatively estimates the global demand for corneal tissue at 12.7 million patients, with only 1-in-70 patients needing tissue being addressed [1]. Approximately 185,000 corneal transplants are performed annually in 116 countries, leaving over 12 million corneal-blind cases worldwide untreated. One of the biggest limitations to corneal transplantation is access to quality donor tissue due to inadequate eye donation services and infrastructure in developing countries. This is compounded by the fact that there is no long-term storage solutions for effectively preserving spare donor corneas collected in countries with a surplus. Eye banking infrastructure requires large amounts of local investment and labour to put in place, however, increased access to tissue can be achieved by development of preservation techniques to increase corneal storage times and allow for global shipping at ambient temperature. This preservation will also allow storage on hospital shelves, for use in emergencies, where waiting for donor corneas is not possible.

METHODS:In this study, we used a novel drying technique (low temperature vacuum evaporation) to preserve human corneas collected in the US and UK, that were deemed unsuitable for transplantation due to problems with their endothelial layer. We assessed weight, thickness, transparency, cell viability, cell membrane permeabilisation, ECM content and structure, comparing to non-dried donor corneas. A subcutaneous implantation model was performed in rats to assess biocompatibility and cell integration of the dry corneas. Clinical suitability was assessed through market access research targeting corneal consultants in the UK.

RESULTS:The dried corneas were comparable to non-dried donor corneas in all investigated aspects except cellular viability, rendering them devitalised. When implanted subcutaneously in rats, the dried cornea was well tolerated, with cellular migration into the matrix and no visible immune rejection. We spoke to 12 corneal consultants, at 7 different hospitals, all gave positive feedback regarding future use and potential clinical indications.

DISCUSSION & CONCLUSIONS:Our preservation technique provides an easy-to-manufacture, non-viable, dehydrated, cornea suitable for a range of clinical indications and tectonic support in emergency situations. It can be stored on the shelf in hospitals for over 2 years and can be shipped at ambient temperatures worldwide, relieving the global shortage of corneal tissue.

ACKNOWLEDGEMENTS:Funding provided through an MRC Confidence-in-Concept award, EPSRC Impact Acceleration Account Award and Innovation and Knowledge Centre (IKC) for Medical Technologies Proof-of-Concept award.

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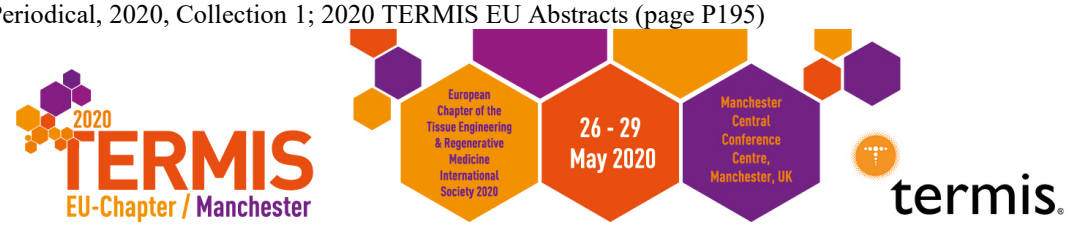
Keywords: Decellularised matrices, Trauma / surgery and rehabilitation



Assessing the Therapeutic Potential of Corneal Mesenchymal Stem Cells for Ocular Surface Disorders

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INTRODUCTION:Following severe mechanical or microbial insult to the cornea, the destructive acute inflammatory phase can activate the transformation of keratocytes to scar-forming fibroblasts, causing significant corneal opacity. Although blindness can be reversed with corneal transplant, excessive inflammation can lead to surgery waiting times of up to 18 months. Corneal stromal mesenchymal stem cells (C-MSCs) have previously been identified to possess potent anti-inflammatory properties [1], and their incorporation into a regenerative medicine therapy could provide an effective treatment to combat acute inflammation and reduce the wait for surgery. The exterior anatomical location of the cornea is also ideal for topical cell delivery. To ensure success in terms of clinical translation, a thorough understanding of any phenotypic or genotypic changes over cell passages is required, in addition to assessment of their survival, behaviour and anti-inflammatory response when placed in a microenvironment as toxic as an injured ocular surface.

METHODS:C-MSCs were isolated from human corneoscleral rims. To identify changes in C-MSCs over passages, flow cytometry and immunocytochemistry were utilised for phenotypic analysis, PrestoBlue assays for cell viability, RT-PCR for genotypic assessment and growth behaviour was calculated. An in vitro inflammatory environment was created by incorporating inflammatory factors into the medium. Cell response was analysed through flow cytometry, immunocytochemistry, ELISAs, and Live/Dead, PrestoBlue and cytotoxicity assays.

RESULTS:C-MSCs maintained an MSC phenotype from passage 4 to 10, with no significant difference in MSC marker or gene expression, viability or growth rates, indicating a freedom for use in a therapy. Inflammatory cocktail addition reduced cell survival, however remaining C-MSCs demonstrated increased anti-inflammatory potency and eluded to a more homologous population. These results provide insight into C-MSC response following topical application to an injured ocular surface, and the potential benefits of pre-treating the cells to augment therapeutic capacity with increased regulation.

DISCUSSION & CONCLUSIONS:Here we present initial steps into the use of C-MSCs in a topical cell therapy for ocular surface disorders, demonstrating the maintenance of C-MSC phenotype and genotype over time, the increased anti-inflammatory potency of C-MSCs when treated with inflammatory factors, and the potential formation of a more homologous population. These results provide an insight into the development of a C-MSC therapy with increased potency and greater scope for regulation.

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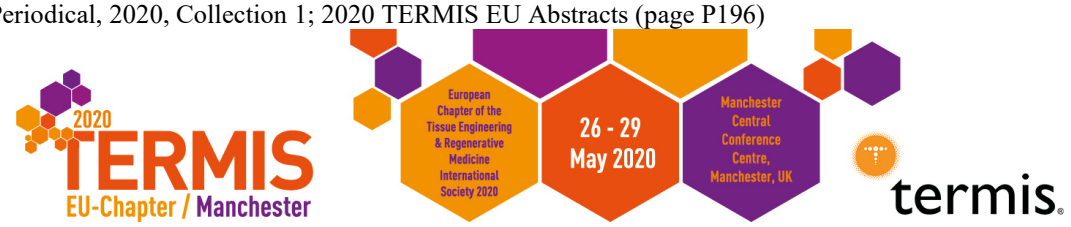
Keywords: Cell therapy, Multipotent (mesenchymal) stem cells



Bipolar cells, a safe and functional target for ectopic expression of human Rhodopsin

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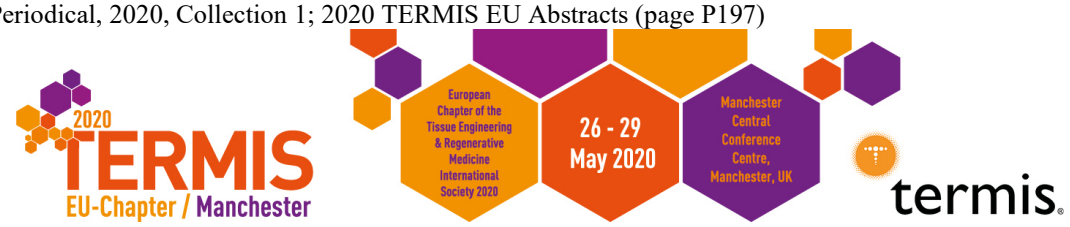
INTRODUCTION:Optogenetic therapies are promising therapeutic areas in the field of vision restoration. Within certain retinal diseases such as retinitis pigmentosa, photoreceptors are damaged and eventually degenerate. However, cells residing within the inner nuclear layer (INL) and ganglion cell layer remain relatively intact, making cells within these layers potential targets for optogenetic treatment. Within the INL, bipolar cells are ideal targets as they maintain some of the previous downstream retinal architecture, allowing for processing to occur within the retina. The delivery method of choice for optogenetic therapies are adeno-associated viruses AAV's, however, previous data has shown the outer nuclear layer to be susceptible to thinning when using AAV's. With this information, it is important to clarify any potential detrimental effect that ectopic Rhodopsin expression may have on the retina when targeting bipolar cells with an AAV. This study aims to determine whether bipolar cells are a safe and functional target for future retinal optogenetic therapies.

METHODS:Adult C57BL/6 GRM6Cre were used as "non degenerate retinal models" or bred onto an Rd1 background with C3H/HeNCrl mice to create the "degenerate retina". Mice were intravitreally injected in both eyes with 2.5µL of AAV vector and electrophysiological recordings conducted using a 256 electrode array. Retina's were immunolabelled for either mCherry or PKCa, and apoptotic cells labelled utilising the Click IT Plus TUNEL assay. mRNA expression of mCherry and PKCa was also assessed via qPCR and retinal thickness was measured via ocular coherence tomography using the Pheonix Micron 4 system.

RESULTS:GRM6Cre treated mice were found to possess widespread transgene expression when intravitreally injected with the AAV2 package, and expression was found to be localised to bipolar cells. Further to this, treated mice were found to display light responses to a variety of light intensities compared to negative controls which displayed little to no light responses. Rhodopsin treated retinas did not display any changes in total retinal thickness or INL thickness within both degenerate and non-degenerate retinas'. Alongside this, ectopic rhodopsin expression within bipolar cells did not cause a difference in specific bipolar cell marker expression or cause any significant increase in levels of apoptosis within the INL.

DISCUSSION & CONCLUSIONS:The GRM6Cre mouse line used within this study has allowed for specific targeting of bipolar cells, and utilising this mouse line, we have shown that bipolar cells are not only a safe target for ectopic Rhodopsin expression, but when transduced to express Rhodopsin, reproducible light responses can be found.

Keywords: Eye, Gene therapy



Impact of Exosomes Released by Different Corneal Cell Types on Human Corneal Epithelial Cells Wound Healing

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INTRODUCTION:The integrity of the cornea is crucial for proper light transmission to the retina. Because of its position at the outer surface of the eye, it is subjected to traumas that may alter the vision quality. Corneal wound healing involves communication between the 3 layers of the cornea (epithelium, stroma and endothelium) that is in part ensured by exosomes. Exosomes are small extracellular vesicles which diameter varies between 30 and 150 nm. The goals of the present study are: i) to characterize the exosomes released by human corneal epithelial cells (hCECs), human corneal fibroblasts (hCFs) and human corneal endothelial cells (hCEnCs), ii) to compare their secretion level during wound healing, iii) to analyse their potential to alter the genetic and proteomic profiles of these cells and iv) to investigate how these exosomes influence the healing of wounds by hCECs.

METHODS:HCECs, hCEFs and hCEnCs were isolated from the cornea of normal human eyes and cultivated in appropriate conditions. Exosomes released by confluent cell cultures, or after scratching, were isolated and quantified. Their size distribution was determined by Dynamic Light Scattering. Exosomes released by confluent hCECs, hCFs and hCEnCs were separately deposited on wounded hCECs at concentrations of 10^6, 10^8 and 10^{10} exosomes/ml. Cultures were photographed 0, 5 and 7 hours after scratching to monitor the healing process. Gene profiling and kinase arrays were conducted in order to verify the impact of the exosomes on the gene expression profile and kinases activation.

RESULTS:The diameters of exosomes released by hCFs, hCENCs and hCECs are 164,49 nm, 102,71 nm and 59,83/100,38 nm (2 exosome populations were observed for hCECs), respectively. HCEnCs significantly released more exosomes per cell ($3.7x10^5$ at confluence and $2.0x10^5$ during wound closure) compared to hCECs ($3.9x10^2$ at confluence and $7.5x10^3$ during wound closure) and hCFs ($5.0x10^4$ at confluence and $1.6x10^5$ during wound closure). For the wound healing assay, scratched hCECs that received 10^{10} hCECs exosomes /ml and 10^{10} hCEnCs exosomes/ml were completely closed after 7 hours compared to controls where 37,9 % of the wound was remaining. The concentrations of 10^6 and 10^8 hCECs exosomes/ml also considerably accelerated the healing process.

DISCUSSION & CONCLUSIONS:Exosomes released by hCECs and hCEnCs clearly accelerated wound closure of hCECs in vitro. Further functional characterization of exosomes released by corneal cells is essential to shed light on the mechanisms of wound repair and may provide a new therapeutic approach to accelerate wound healing.

Keywords: Extracellular vesicles, Wound healing

Cell carriers suitable for culturing human corneal epithelial cells for the production of tissue-engineered corneas: a comparative study

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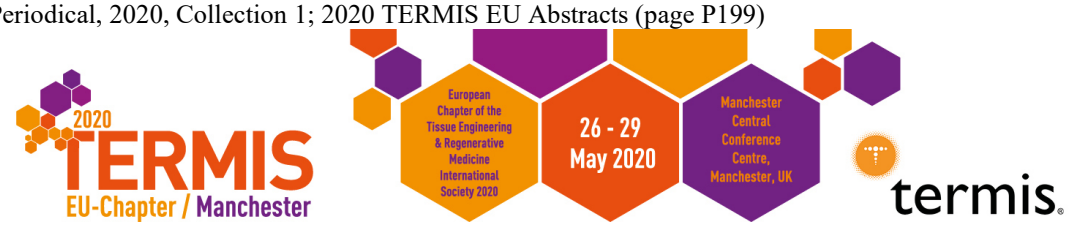
INTRODUCTION:The culture of human corneal epithelial cells (HCECs) has many clinical and research applications in the treatment of corneal pathologies such as limbal stem cell deficiency. In this case, it is therefore necessary that culture conditions allow a good proliferation of HCECs and prevent massive terminal differentiation to ensure the long-term regeneration of the corneal epithelium. For the production of tissue-engineered corneas, several carriers can be used to cultivate and graft HCECs: human amniotic membranes (HAMs), collagen gels (CGs), human fibrin gels (HFGs) and human corneal stromas (HCSs) produced by the self-assembly approach. Our goal is to determine which of these four carriers is the most effective at ensuring HCECs proliferation and regeneration of a pluri-stratified corneal epithelium, a prerequisite for grafting of the tissue-engineered cornea.

METHODS:HCECs were seeded and grown as monolayers on four different cell carriers (HAMs, CGs, HFGs and HCSs) until 1 day post-confluence. Human tissue-engineered corneas (hTECs) were also produced by the self-assembly approach using the same populations of HCECs and wounded with a 8 mm biopsy punch. Wounded HTECs were then deposited on the different carriers and corneal wound healing monitored until complete closure of the wounds. Histological and immunofluorescence analyses were conducted on monocultures and healed hTECs to assess: the presence of a sub-population of corneal epithelial stem cells (ABCG2/ABCB5, ΔNp63α and K15), the epithelial layer integrity (Na+/K+-ATPase, K3/12 and ZO-1), and the expression of basal membrane components (collagen IV and laminin V).

RESULTS:The rate of wound closure was increased when wounded hTECs were grown on HAM. The rate of wound closure was similar when wounded hTECs were grown on HCSs, HFGs and GC. For the various conditions, histological sections showed the presence of a typical corneal epithelium in the centrally wounded area.

DISCUSSION & CONCLUSIONS:The hCECs' proliferative potential was affected by the nature carrier as we noted a marked increase in the wound healing dynamic when wounded hTECs were deposited on the HAM. This study provide valuable data on the culture conditions required to yield hCECs with the best proliferative and differentiation properties in order to ensure the production of hTECs of an optimal quality. From a translational point of view, this study constitutes a major step forward in the production of a human corneal substitute that will be of a sufficient quality to be used in patients as a graft for corneal replacement in a near future.

Keywords: Eye, Wound healing



Generation of Novel Cornea Substitutes with Improved Biomechanical Properties Based on Lipid Nanoparticles

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INTRODUCTION:Development of an efficient substitute of the human cornea is dependent on the availability of an adequate biomaterial. In this regard, most biocompatible biomaterials are not biomechanically adequate. The recent development of biocompatible lipid nanoparticles (LNP) able to load different types of growth factors (1,2) opens the possibility of generating novel scaffolds with improved biological functions, although the biomechanical properties of these scaffolds have not been determined to the date. In the present work, we have developed novel cornea stroma substitutes based on biomaterials containing lipid nanoparticles.

METHODS:We first generated a fibrin-agarose biomaterial using human plasma obtained from healthy donors and type VII agarose at a final concentration of 0.1%. These were considered as control cornea stroma substitutes. Then, we generated novel cornea stroma substitutes containing LNP previously developed as nanocarriers for different growth factors. These LNP were used at increasing concentrations (10, 100, 300 and 1000 µg/ml). To determine the effect of LNP on the biomechanical properties of the cornea stroma substitutes, we carried out several biomechanical analysis in the traction mode using an Instron analyzer. Results were assessed in triplicates.

RESULTS:Analysis of control cornea stroma substitutes devoid of LNP showed an average Young modulus of 0.12±0.04 MPa. These results were not significantly different to substitutes containing LNP at the lowest concentrations (0.08±0.02, 0.10±0.05 and 0.07±0.01 MPa for 10, 100 and 300 µg/ml of LNP, respectively). However, the use of high concentrations of LNP showed significantly higher values of the Young modulus of the bioartificial cornea stroma substitutes (0.53±0.34 MPa at a concentration of 1000 µg/ml of LNP).

DISCUSSION & CONCLUSIONS:The use of LNP could contribute not only to improve the biological functions of the cornea scaffolds, but also to enhance the biomechanical properties of the cornea stroma substitutes by increasing the stiffness of these substitutes. These results support the use of LNP and suggest that bioengineered human corneas containing these LNP could be biomechanically adequate once grafted in vivo.

ACKNOWLEDGEMENTS:Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i) from the Spanish Ministerio de Ciencia, Innovación y Universidades (Instituto de Salud Carlos III), Grant FIS PI17/0391 (cofinanced by FEDER funds, European Union) and the Spanish Agencia Estatal de Investigación and by FEDER funds, within the project RTC-2017-6696-1

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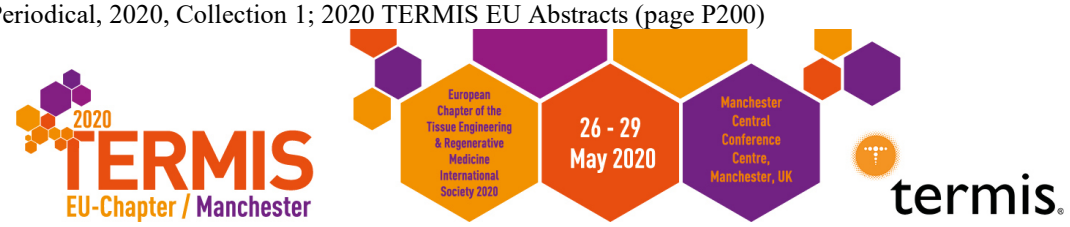
Keywords: Nanomaterials (inc graphene), Eye



Validation and assessment of an antibiotic decontamination manufacturing protocol for vacuum-dried human amniotic membrane

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INTRODUCTION:Elective caesarean section-delivered amniotic membrane (CAM) contains microbial bioburden that requires decontamination before transplantation. This study assessed the decontamination ability of the Tereo® manufacturing process to produce the commercial dry amnion product Omnigen® (NuVision Biotherapies, UK), and investigated the potential antimicrobial properties of long-term stored Omnigen.

METHODS:Bioburden of 10 fresh CAM were assessed using in vitro microbial culture. Subsequently, the Tereo process was assessed for decontamination ability. Five CAM were artificially loaded (106 CFU/mL) with Staphylococcus epidermidis at three different stages of processing; i) before the full manufacture processing; ii) prior to antibiotic treatment; and iii) immediately before drying, and resulting products assessed by microbial cultures. The long-term stability and antimicrobial activity of 10 Omnigen was assessed after 2.3 year storage; antibacterial activity of non-antibiotic treated CAM compared to Omnigen was evaluated using MIC/MBC, and disc diffusion assays against Meticillin-resistant Staphylococcus aureus, Meticillin-resistant S. epidermidis, Escherichia coli, Pseudomonas aeruginosa and Enterococcus. faecalis.

RESULTS:Bioburden of CAM was found to be very low. The Tereo process was highly efficient at removing bioburden introduced at any stage of processing. The combined process was comparable to a sterilising process. CAM not treated with antibiotics was shown not to be antimicrobial. However, Omnigen demonstrated effective antibacterial capacity against all bacteria tested. Antimicrobial activity of Omnigen was not reduced after 2-year storage.

DISCUSSION & CONCLUSIONS:Antibiotic decontamination is a reliable method for sterilization of CAM and the resultant antibiotic reservoir is effective against gram-positive and –negative bacteria. The research suggests, amnion products manufactured without the use of antibiotics possess little antimicrobial activity. However, Omnigen may be useful in the treatment strategy of microbial keratitis.

ACKNOWLEDGEMENTS:This study was funded by The Authority for Research, Science and Technology of the Ministry of Higher Education and Scientific Research-Libya (Scholarship 404/2013), an Innovate UK Biomedical Catalyst Feasibility Award (132044) and the Defence Science & Technology Laboratory, UK.

Keywords: Scaling up & manufacturing, Biomaterials

Consistent tensile testing of electrospun nanofibers

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INTRODUCTION:Nanofibrous mats find uses in tissue engineering, among other applications fields like drug delivery and energy harvesting. In all cases, the mechanical properties of the constituent fibers is very important. Previous works have focused on the measurement of tensile properties of isolated fibers, requiring the development of specific methods.[1] We have applied standard testing tools to the determination of tensile properties of aligned fiber mats containing many of fibers, from which the properties of single fibers can be obtained consistently and robustly. PCL fibers are particularly difficult to handle as they are easily deformed, so they served as an ideal test to our method.

METHODS:Mats of well aligned electrospun PCL fibers were produced by electrospinning a 12 wt. % solution in a 3:1(v/v) chloroform-methanol mixture on a rotating wire-mandrel.[2] The fibers were glued to a paper frame, which was then set in a Dynamic Mechanical Thermal Analysis Analyzer, where the force-displacement relationship in tension was obtained in static-force ramp mode.

RESULTS:Several data sets were collected from separate electrospinning experiments. From the DMA data, the force was converted to stress (for a single fiber) by dividing by the aggregate cross-sectional area of all the fibers in each sample (sample volume divided by fiber length); while the strain was obtained by dividing the displacement by the original fiber length. The stress-strain curves were found very consistent across different samples and experiments. The Young modulus falls in the range of about 403 MPa. The yield strain is about 6%, and the stress and strain at break 130 MPa and 65% respectively.

DISCUSSION & CONCLUSIONS:We have demonstrated a robust method to establish the tensile properties of single polymeric nanofibers. These properties can be extrapolated to those of fibers in randomly oriented mats (not tested), as these are like the fibers in aligned arrangement.

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Keywords: Additive manufacturing, Nanomaterials (inc graphene)

Cultivating Skin the Old-Fashioned Way: Could the Future Be Knitted?

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INTRODUCTION:Replacement of skin by tissue engineering remains a challenge, despite developments in regenerative medicine. Porous, 3D scaffolds mimic the structural properties of skin's extracellular matrix (ECM) to direct fibroblast cell growth.[1] Polymeric scaffolds, e.g. electrospun membranes, are however, often too thin and delicate to engineer full thickness skin.[2] Biocompatible textile fibres and fabrics have inherent porosity to support cell and nutrient infiltration, whilst microscale fibres can direct cell growth.[3],[4] The potential of knitted textiles as durable scaffolds for skin tissue engineering is investigated.

METHODS:Rib and purl knitted fabrics (RPKFs) were produced from yarns containing protein, manmade or natural polymer-based fibres. Mechanical properties (tensile and tear) of RPKFs (sample dimensions 200 x 50 x 1 mm and 80 x 68 x 1 mm) were evaluated using Instron 2519-107 (strain rate 200 mm/min) and SDL Atlas M008HE digital Elmendorf instruments (64 N load), under standard conditions (21±1°C, 65±1% humidity), compliant with British Standards EN ISO 13934-1:1999 and 13937 1:2000. Fibre microstructure pre/post-sterilisation by autoclave (steam at 126.5 °C) was assessed by Scanning Electron Microscopy (SEM). Fibroblast viability and cytotoxicity on RPKFs were evaluated after 3 days using the Live/Dead assay.

RESULTS:Initial results showed higher tensile and tear strengths perpendicular to fabric selvages with acrylic and lyocell fibre yarns. SEM revealed that the structure and size of all fibres were unchanged following sterilisation. Live/Dead assay revealed that RPKFs were non-cytotoxic after 3 days, with lyocell fabrics exhibiting more viable cells.

DISCUSSION & CONCLUSIONS:Development of standards for more accurate RPKF mechanical testing is required as their use in technical textile applications increases.[5] RPKFs were undamaged during sterilisation and appeared to be non-cytotoxic to the cells. Natural polymer-based RPKF demonstrated higher cell density and viability, which may indicate its potential for tissue regeneration. Fibroblast cell viability, proliferation and collagen production data beyond 3 days will also be presented.

ACKNOWLEDGEMENTS:The authors thank the support of the UK Engineering and Physical Sciences Research Council.

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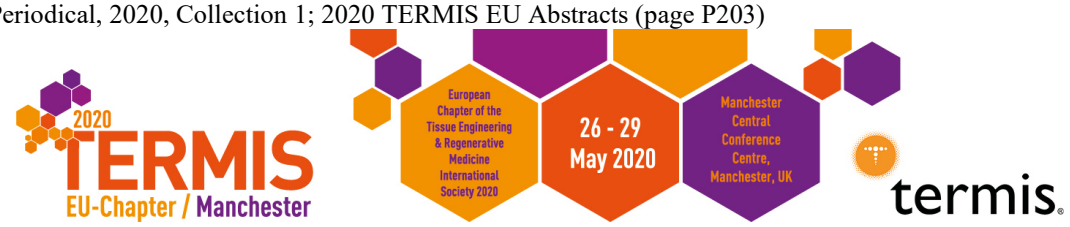
Keywords: Skin, Polymers - natural / synthetic / responsive



Textile reinforcement for a biohybrid venous valve implant

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INTRODUCTION:One of the most common adult diseases in the western population is chronic venous insufficiency (CVI) mostly being found in the legs. The prevalence of CVI is 3-5 % in the western world, therefore it has a considerable socio-economic significance. Insufficiency of the venous valves disrupts the return of blood to the heart and leads to a local overpressure in the venous system of the legs. Symptoms of CVI are dilated veins, varicose veins, heavy and painful legs with swelling, skin and tissue changes and in the final stage to chronic lower leg ulcers. Currently only symptomatic treatments are available. Due to the special coagulation situation in the venous flow region current reconstructions of the venous valve function has not been successful. The aim of the project is to restore the venous valve function by means of a biohybrid implant.

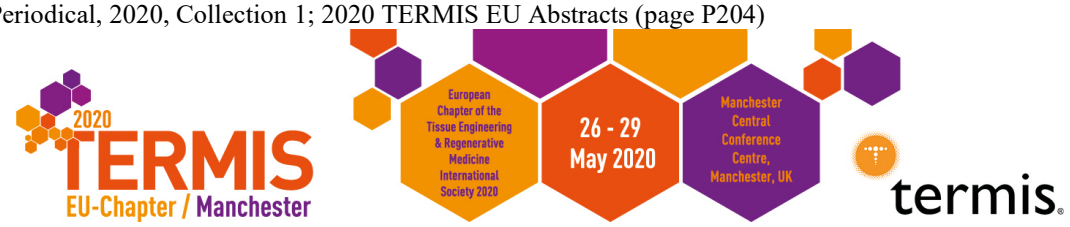
METHODS:The approach in this projects is the use of two textile-reinforcement structures. A biodegradable magnesium stent is used for the overall mechanical properties. A warp-knitted textile reinforcement structure is used for sealing and cell settlement. The supporting framework in form of the stent is manufactured by different braiding technologies i.e. manual and machine manufacturing. Different stent designs are developed and produced using biodegradable magnesium alloy. The stent designs are being tested regarding incorporation of a textile valve structure, crimp ability and radial force in comparison to commercial venous stents. The textile reinforcement structure for the valve structure is manufactured by warp-knitting. Within the project the warp-knitted mesh is optimized regarding pore size, porosity and mechanical requirements.

RESULTS:Four different stent designs were successfully manufactured and validated. Due to variation in the geometry, filament count and braiding angle significant differences regarding the radial force are realised.

DISCUSSION & CONCLUSIONS:The data illustrates that the biodegradable magnesium wire is suitable to manufacture different new stent designs for venous valve implants. Furthermore, it is shown that the incorporation of textile reinforced valve leaflets comparable to native venous valves is possible.

Acknowledgements:The Project “BioV²alve” is supported by the European Regional Development Fund North Rhine-Westphalia (EFRE.NRW).

Keywords: Fibre technology, Cardiovascular



Trabecular meshwork cells infiltrate scaffolds fabricated by cryo-electrospinning

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INTRODUCTION:The trabecular meshwork (TM) is located around the base of the cornea and can be described as a biological sieve.[1] It is essential for maintaining the eye's intraocular pressure (IOP), but age and disease reduces TM cellularity, which can lead to blockages of the porous tissue, resulting in an increase in IOP and permanent loss of peripheral vision.[2] This type of vision loss is widely known as glaucoma. This study investigated the infiltration of human TM cells cultured on biomaterial sieves with varying thickness as a potential new substrate for TM regeneration.

METHODS:Poly(ε-caprolactone) (PCL; 12%w/v) was dissolved in 1,1,1,3,3,3,-hexafluoroisopropanol and stirred continuously for 48 hours. 0.001% w/v rhodamine was added prior to electrospinning. Spinning parameters applied: needle voltage +15kV, collector voltage -4kV, flow rate 1 ml/h, distance 17cm and rotating mandrel 100RPM. On separate runs, the mandrel was packed with dry ice to initiate ice crystal formation on its surface during fibre collection and quickly vacuum-dried for water removal without structural collapse.
Scaffolds were mounted in 24-well CellCrowns, disinfected in 70% ethanol, washed in sterile phosphate buffered saline and pre-soaked in culture medium overnight. NTM5 cells (2x104) were seeded and cultured for 7 days. Scaffolds were fixed in 10% neutral buffered formalin, stained with FITC-Phalloidin (1:40) and DAPI (1:1000) and imaged by confocal microscopy. Images were analysed using Fiji ImageJ software (v2.0.0).

RESULTS:Solutions electrospun onto a dry ice-containing mandrel resulted in thicker scaffolds compared to those collected on a standard mandrel (64.89±11.47µm cryo-PCL; 42.43±4.41µm PCL) and there was no change in fibre diameter (0.60±0.12µm cryo-PCL; 0.64±0.14µm PCL). Confocal imaging demonstrated adhesion of NTM5 cells on both scaffolds, with cells infiltrating to a depth of 32.12µm on the cryo-PCL, but no observed migration on PCL after 7 days.

DISCUSSION & CONCLUSIONS:Cryo-spinning yielded thicker scaffolds compared to standard electrospinning with no effect on fibre diameter. Furthermore, unlike the regular scaffolds, cells penetrated the cryo-scaffolds, as a greater pore size had been achieved, which allowed their infiltration. Future studies will focus on optimising the thickness and porosity of cryo-scaffolds to better mimic the natural tissue, where healthy human tissue ranges between 110-130µm in thickness.[3]

ACKNOWLEDGEMENTS:Special thanks to the Crossley-Barnes bequest and the Healthcare DTN for supporting this research.

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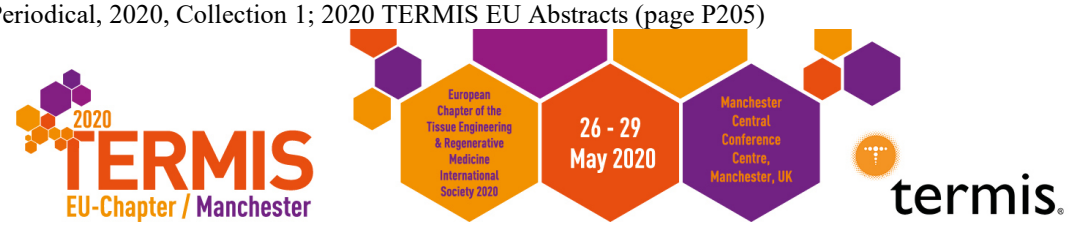
Keywords: Eye,



Developing human opsins for optogenetic control of G-protein signalling in human iPSC-derived cells

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INTRODUCTION:Optogenetics is a powerful technique allowing temporally and spatially precise control over cellular function. Human opsins (including rhodopsin and melanopsin) are potentially important optogenetic actuators for regenerative medicine. As native human proteins, they have lower immunogenic risk than alternatives from other species. Moreover, they are light-activated G-protein coupled receptors allowing them to provide control over this influential aspect of cell physiology. G-protein signalling cascades are targeted by 35% of approved pharmaceuticals, and provide functions of significant importance in regenerative medicine (e.g cardiac pacemaker function and visual transduction). We set out to establish human opsins as a method of achieving optogenetic control over G-protein signalling in human iPSC-derived cells.

METHODS:Expression cassettes containing either human rhodopsin (genbank: NM_0.0059.3) or melanopsin (genbank: NM_033282) were stably integrated into the human dermal fibroblast-derived induced pluripotent stem cell (iPSC) line SW171A using CRISPR-mediated HDR into the AAVS1 safe harbour site. Alternatively, Floxed-AAV2 vectors containing CMV-driven opsin were co-transfected with AAV6-Cre into iPSCs-derived progenitors and terminally differentiated cells. iPSCs were differentiated to neuronal and cardiac fates using modified defined differentiation protocols[1][2]. Optogenetic control of G-protein activity was assayed using luminescent second messenger assays, including GloSensor and Aequorin.

RESULTS:Gene transfer of human opsins (rhodopsin and melanopsin) as optogenetic actuators was achieved in both iPSCs and progenitors. Suitable AAV vectors transduced human iPSC-derived progenitor populations with good efficiency to drive strong expression of human opsins in terminally differentiated cells. In addition, transgenes driving expression of human rhodopsin and melanopsin under the CAG promoter were successfully introduced to the AAVS1 safe harbour site of human iPSCs using CRISPR-mediated gene transfer. The resultant lines showed stable expression of the two human opsins. Both human rhodopsin and melanopsin drove time-delimited activation of G-protein signalling at relatively low light intensities in live cells.

DISCUSSION & CONCLUSIONS:We provide the first application of human opsins to iPSCs and their differentiated derivatives. Good expression is possible both by CRISPR-mediated transfer to iPSC and by transduction of progenitor cells with recombinant AAV and subsequent terminal differentiation. The ability of these human opsins to allow-light dependent control over cell physiology at low light levels and with good temporal resolution has great potential for numerous applications in regenerative medicine.

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Keywords: Induced pluripotent stem cells

Creation of a linear covalently-closed Hairpin Expression Construct capable of effective gene transfer within a Gene Activated Matrix (GAM)

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INTRODUCTION:By acting as a template, scaffold biomaterials possess significant influence over the behaviour and fate of cells in contact with it. As such, GAMs – loaded with therapeutic genetic payloads, hold significant potential as regenerative devices for tissue regeneration. However, current GAMs are limited by the vehicle/vector for which therapeutic DNA is carried and delivered in-situ (e.g. plasmid (pDNA)), specifically the efficiency and selectivity of gene transfer, and subsequent gene expression at the site of interest. An alternative expression construct is therefore proposed – A Linear covalently-closed Hairpin Expression Vector, of which literature suggests has the capacity for two-four fold greater gene expression as well as higher transfection efficiencies (1, 2).

METHODS:A reporter plasmid expressing GFP (pCAG-GFPd2) was first linearized using the restriction enzyme (MunI). Thus eliminating the unnecessary bacterial elements (ampicillin resistance & origin of replication (Ori)) whilst exposing the gene expression cassette (promoter sequence, coding sequence, termination signal). Annealing and ligation steps followed, using T4 DNA Ligase (25U/ml), in the presence of a 5' unphosphorylated hairpin oligonucleotide (in 200X Excess). Through 5'-3' exonuclease resistance (T7 DNA Polymerase- 150U/mg), confirmation of successful hairpin attachment was attained. Isolation was performed via Anion Exchange Chromatography and ethanol precipitation.

RESULTS:Two different Linear covalently-closed Hairpin Expression Constructs were generated through the outlined method. Future work will incorporate these constructs within a previously developed mineralised agarose hydrogel GAM, compare the transfection efficiencies of the generated expression vectors against previously researched plasmid based gene delivery systems. Moreover, methodologies such as nanoparticle cation-DNA complexation and Polyethylenimine (PEI) integration will be evaluated with a view for further enhancement of transfection and gene expression characteristics.

DISCUSSION & CONCLUSIONS:In summary, a multi-vector GFP reporter gene DNA library has been generated with a view for use within a GAM biomaterial strategy. Through such a combination of tissue engineering techniques and the above molecular engineering (to create advanced nucleic acid payloads), it is hoped the capacity to provide biocompatible 3D niches that induce or support tissue integration and regeneration will be greatly improved.

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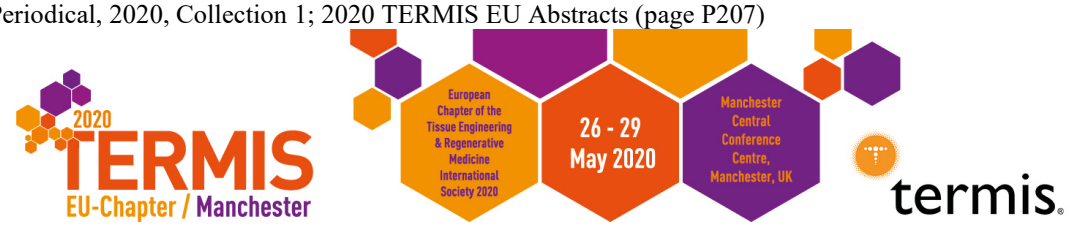
Keywords: Gene therapy, Biomaterials



Development of hybrid collagen and alginate hydrogel for osteochondral regeneration

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INTRODUCTION:A scaffold for osteochondral repair that is able to regenerate bone and cartilage is still required [1]. Recently, it has been shown that matrix stiffness is a key determinant of mesenchymal stem cells differentiation [2], suggesting that the modulation of mechanical properties of the scaffold would be important in guiding stem cell differentiation either into cartilage or bone lineages. In this study, we developed a hybrid collagen and alginate hydrogel with tuneable stiffness.

METHODS:By varying the concentration of the crosslinker hydrogels of different stiffness were obtained. Viability of ovine mesenchymal stem cells (oMSCs) encapsulated into hydrogels of different stiffness was evaluated by calcein AM/ethidium homodimer staining and LDH assay. To evaluate cell proliferation within the hydrogels oMSCs were stained using carboxyfluorescein succinimidyl ester (CFSE) Celltracker. Moreover, nutrient uptake within the 3D culture was measured using fluorescent glucose probe. After 7 days of culture, cellular morphology of oMSCs within the hydrogels was assessed using both wheat germ agglutinin (WGA) and phalloidin staining.

RESULTS:Scaffolds suitable for osteogenic differentiation (19kPa) [3] and chondrogenic differentiation (3.9kPa) [4] were successfully obtained by modifying formulation parameters. Live/dead and CFSE staining showed that cells were viable and proliferating within both formulations. It was possible to assess that none of the formulations was cytotoxic as the LDH release in the media was comparable to the 2D monolayer. Furthermore, both hydrogels assured good nutrients transport as demonstrated by glucose uptake with no significant difference between the 2D and the 3D culture. Interestingly oMSCs retained a rounded chondrocyte morphology within both the 3.9kPa and 19kPa hydrogels.

DISCUSSION & CONCLUSIONS:Data collected suggest that the hydrogel itself, without any exogenous stimuli, might have an effect on oMSCs commitment.

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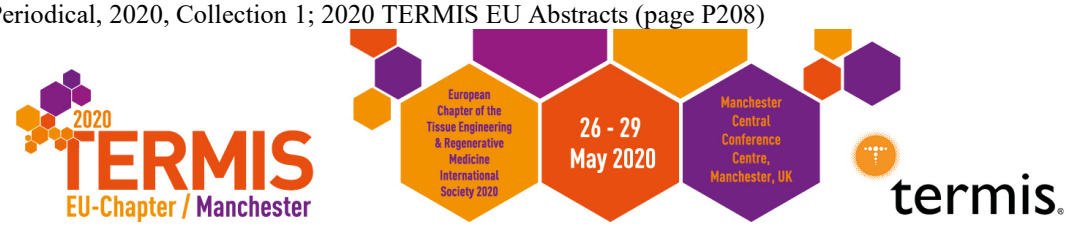
Keywords: Biomaterials, Cartilage / joint and arthritic conditions



Gelatin-PEG hydrogels loaded with microRNA-nanoparticle complexes for enhanced osteogenesis of mesenchymal stem cells

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INTRODUCTION:Despite extensive research in bone tissue engineering, clinical translation of this practice still has several limitations. Strategies incorporating mesenchymal stem/stromal cells (MSCs) in biomimetic hydrogels have huge potential in bone tissue regeneration but are limited by their differentiation efficiency. The ability of microRNAs (miRNAs) to modulate osteogenic differentiation of MSCs is emerging as a powerful approach to enhance bone regeneration. However, miRNA selection and delivery must be improved to achieve this end. By combining pro-osteogenic miRNAs, nanoparticles as transfection agent and MSCs within a hydrogel, this project aims to design a simple system to enhance osteogenesis of MSCs and promote bone regeneration.

METHODS:Human bone marrow-derived MSCs were encapsulated in a gelatin norbornene and polyethylene glycol (PEG) dithiol photo cross-linked hydrogels. Hydrogels were fabricated in bulk form and a facile pipette tip-based microfluidic device was used to form microgels containing 2.5 million cells/ml. These were assessed for cell viability (live dead staining), morphology (actin staining) and osteogenic differentiation potential (qPCR and mineralization). Screening of pro-osteogenic miRNAs was conducted based on alkaline phosphatase activity (ALP) at day 7 and mineralization at day 21. Porous silicon nanoparticles (pSiNPs) were assessed for cell viability and cell internalization as a potential miRNA carrier.

RESULTS:Hydrogels with a storage modulus of ~2 kPa were selected as a base for the system. These were stable in-vitro over 21 days and supported high viability (>85%) of encapsulated MSCs in both bulk and microgel formats. Cell morphology indicated spreading and interaction with the hydrogel matrix. In the presence of osteogenic medium, MSCs encapsulated in both bulk hydrogels and microgels showed significantly increased RUNX2 and ALP gene expression and enhanced mineralization. Screening of miRNAs identified enhanced osteogenesis in the presence of miRNA-96a, 125b, 200a and 375 and the optimum combinations of these candidates were determined. MSCs treated with pSiNPs retained excellent cell viability and ability to undergo osteogenesis, and internalized pSiNPs in a concentration-dependent manner.

DISCUSSION & CONCLUSIONS:Our findings suggest that gelatin-PEG bulk hydrogels and microgels form an effective basis for an osteogenic hydrogel. We have identified a set of miRNAs that promote osteogenesis and shown that pSiNPs hold a significant promise as a vector to deliver these miRNAs. By combining these elements, there is future potential for this novel miRNA-complexed NP-hydrogel hybrid system to improve MSC-mediated bone regeneration. Furthermore, the injectable nature of the microgel format provides potential for the utility of this one pot system in minimally invasive in-vivo applications.

Keywords: Multipotent (mesenchymal) stem cells, Gene therapy

Tissue biomimetic Collagen-Hyaluronan Hydrogels with simultaneous covalent Cross-linking and Collagen Fibrillogenesis

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INTRODUCTION:Collagen (Col) and hyaluronan (HA) are biopolymers broadly used in tissue engineering thanks to their biocompatibility, biodegradability and their interaction abilities with cells [1 2]. For quite some time Col/HA composite hydrogels have been developed to create biomimetic engineered hydrogels synergizing their properties. In this study, Col/HA hydrogels with tunable physical properties were developed to mimic extracellular matrices of human physiological tissues using the enzyme HorseRadish Peroxidase (HRP), thus going through a biocompatible synthesis pathway.

METHODS:Different contents of Tyramine HA derivative (HA-Tyr) were added to a neutralized 0.4% Col solution in a one-pot synthesis. The gelling kinetic and the cross-linking degree of HA-Tyr were modulated by [HRP] and [H_2O_2], respectively. Ultrastructure and physico-chemical properties of hybrid hydrogels were assessed with scanning and transmission electron microscopy, rheological measurements, swelling properties, in vitro enzymatic degradation and DSC. Normal human dermal fibroblasts were 3D-encapsulated in hybrid Col/HA-Tyr hydrogels at 250,000 cells/cm³ and cultured over 1 and 7 days. Metabolic activity and cell morphology were both evaluated at those timepoints.

RESULTS:At low HA-Tyr content, hydrogels exhibited a fibrillar structure resembling that of pure Col hydrogels. Their swelling and degradation properties were also similar to Col. In contrast, the addition of HA-Tyr until the ratio Col/HA-Tyr 1:1 destabilized the hydrogels and lowered their mechanical properties. At high HA-Tyr content, a microfibrillar network was observed beside the banded Col fibrils and mechanical properties increased with the HA-Tyr content, the [HRP] and the [H_2O_2]. These hydrogels were more resistant against enzymatic degradation than pure HA-Tyr and Col hydrogels while keeping a high degree of hydration (≥ 90%). Unlike pure HA-Tyr hydrogels, encapsulation of human dermal fibroblasts within Col/HA-Tyr hydrogels allowed for a high cell viability and proliferation, thereby evidencing the crucial role of Col for cell survival.

DISCUSSION & CONCLUSIONS:Collectively, these results showed that increasing the HA-Tyr content positively impacted the physical properties of hydrogels due to the presence of the microfibrillar network which stabilized the structure. The tunable physical, mechanical and biochemical properties of Col/HA-Tyr hydrogels preserve fibrillogenesis and allow fibroblasts proliferation.
This platform of Col-HA-Tyr hydrogels appears promising for novel tissue engineering applications following a biomimetic approach, e.g. for the intervertebral disc repair.

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Keywords: Composite materials, Interfaces – biological

Hydrogels Promoting Vascularization for Tissue Engineering Applications

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INTRODUCTION:Tissue augmentation is the basis of a range of therapies in modern medicine, incl. soft tissue (e.g. skin reconstruction after trauma or circulation disorders), and hard tissue augmentation (e.g. alveolar reconstruction prior dental implantation). The regeneration of large tissue defects demands the neovascularization of implants used as a scaffold for tissue augmentation. Therefore, it is of utmost importance to promote the generation of vascular networks within such constructs. A promising approach is to use photocrosslinkable hydrogels as matrix for the ingrowth of neo-vessels. In this study, we employ mimicry of placenta as highly vascularized organ. Hydrogels, based on different gelatin (Gel) modifications, incl. Gel-norbornene (Gel-NB)[1] and Gel-allyl glycidyl ether (Gel-AGE)[2] can be crosslinked in situ, enabling the fabrication of complex structures either by extrusion based 3D printing (i.e. bioprinting, in the µm to cm regime) or by two-photon polymerization (2PP, in the nm to mm regime).[3]

METHODS:Different Gel modifications with varied enes and degrees of substitution (DS) were prepared by the conversion of Gel (type A) in DMSO at 50 to 65 °C with either 5-norbornene-2,3-dicarboxylic anhydride or N-hydroxysuccinimide-activated 5-norbornene-2-carboxylic acid (Gel-NBs), or allyl glycidyl ether (Gel-AGE) for 5 to 48 h. Hydrogel formation was performed by photopolymerization using lithium phenyl-2,4-6-trimethylbenzoylphosphinate as photoinitiator and D,L-dithiothreitol or ethoxylated trimethylolpropane tri(3-mercaptopropionate) as crosslinker in aqueous solution (2.5 to 10 wt%) monitored by photorheology.[4]

RESULTS:Depending on the type and degree of modification of Gel, the mechanical properties (shear moduli 10 to 1000 Pa) and swellability (5 to 40 fold) of the resulting hydrogels can be adjusted. By incorporation of placenta-specific factors into the hydrogels vascular network formation with human umbilical vein endothelial cells was observed.

DISCUSSION & CONCLUSIONS:The prepared hydrogels serve as an adjustable material platform to observe the formation of vascular networks. By variation of the parameters, incl. modification, crosslinker, gel content etc. a suitable system is to be found for the 3D fabrication of vascularization promoting hydrogel constructs.

ACKNOWLEDGEMENTS:The financial support by the Austrian Federal Ministry for Digital and Economic Affairs and the National Foundation for Research, Technology and Development is gratefully acknowledged.

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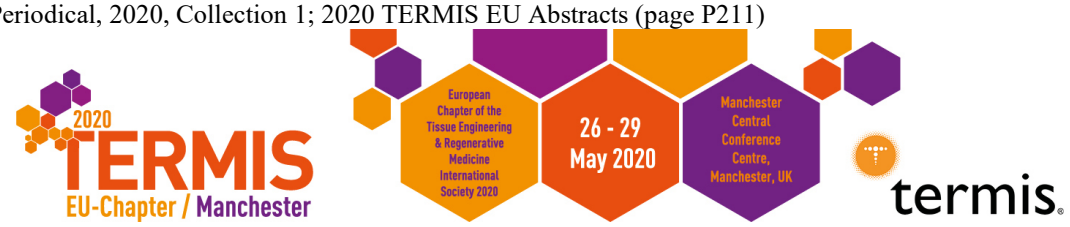
Keywords: Vascular systems / vascularisation and heart, Biofabrication



A novel self-setting hydrogel of silylated chitosan and cellulose for the repair of osteochondral defects in canine model

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INTRODUCTION:To address the clinical issue of osteochondral defects repair, we aimed at developing an injectable, self-hardening and mechanically reinforced hydrogel (Si-HPCH) composed of silanised hydroxypropylmethyl cellulose (Si-HPMC) mixed with silanised chitosan.

METHODS:The in vitro cytocompatibility of Si-HPCH was tested using human nasal chondrocytes (hNC) or human adipose stromal cells (hASC) with a live and dead assay kit. The in vivo biofunctionality of our hydrogel (250 µL) was then determined by implantation in nude mice subcutis. Six different conditions were implanted: Si-HPMC or Si-HPCH alone, Si-HPMC or Si-HPCH mixed with hNC (1 million/ml) and Si-HPMC or Si-HPCH mixed with hASC (1 million/ml). Samples were collected 6 weeks after implantation and characterized by immunohistochemistry with antibodies against type I and II collagen and aggrecan. Si-HPCH was then tested for the repair of calibrated 6 mm-diameter and 5 mm-depth osteochondral defects performed on the medial femoral condyle of twelve 4-year old beagles. 6 defects were filled with Si-HPCH (150 µL) alone or mixed with 1 million/ml of autologous ASC. As negative control, 2 additional defects were left empty. Four months after implantation, histological (safranin O and Movat pentachrome stainings) and immunohistological (type I and II collagen and aggrecan) analyses were performed.

RESULTS:Our data demonstrated that Si-HPCH supports hNC and hASC viability in 3D culture as well as in the subcutis of nude mice as showed by the formation of cell clusters surrounded by a cartilage-like extracellular matrix (ECM). Interestingly, the explants containing hASC mixed with Si-HPMC or Si-HPCH showed the presence of a low but significant number of cells expressing chondrogenic markers. In the canine osteochondral defect model, while the empty defects were only partially filled with a fibrous tissue, defects filled with Si-HPCH with or without autologous ASC, revealed a significant osteochondral regeneration. In empty defects, the repair tissue was thus mainly composed of fibroblast-like cells expressing type I collagen and no chondrogenic marker had been detected. On the contrary, in the defects filled with Si-HPCH, whatever the presence of autologous ASC, the ECM of the repair tissue was positively stained for type II collagen and aggrecan.

DISCUSSION & CONCLUSIONS:Si-HPCH is an injectable, self-setting and cytocompatible hydrogel able to support the regeneration of osteochondral defects when implanted alone or with ASC. Taken together, these data make Si-HPCH a promising candidate for the cell-free regeneration of articular cartilage.

Keywords: Hydrogels and injectable systems, Cartilage / joint and arthritic conditions

Characterization of oxidized alginate – gelatin – calcium hydrogel with controlled mechanical properties: a tumour 3D model for breast cancer study

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INTRODUCTION:The progression of breast cancer has been clinically linked to tissue density and stiffness[1] but there is the need to have more engineered in vitro models expending the knowledge on the effects of matrix stiffness to this process. To fill this gap, we designed a library of hydrogels with known composition and mechanical properties. Alginate is a well-known biocompatible polysaccharide used in tissue engineering while it has the limitation of lacking in cell adhesion properties. To solve this, partially oxidized alginates (OA) were functionalized and hydrogels formed using a stepwise cross-linking strategy: first step OA-gelatin functionalization, second step stiffening with Ca^{2+} to obtain hydrogel stiffness of 1-20 kPa. We show that OA-gelatin-calcium hydrogels provide cell-adhesion sites, as well as controlled stiffness. Hydrogels were further used as bioinks and 3D breast cancer models were obtained to mimic stages of cancer progression.

METHODS:Alginate was oxidized with $NaIO_4$ (aq.) by mechanical mixing in the dark (6h, RT), dialysed against water and freeze-dried. OD was determined by triiodide-starch method; aldehyde groups in OA were measured by hydroxylamine assay; amino groups in gelatin were detected by ninhydrin assay. OA-gelatin-calcium hydrogel were prepared by 24 h gelation of gelatin-OA mixture at RT, followed by 10 mins secondary gelation in calcium chloride solution at 37°C. Stiffness was measured on day 1, 3 and 5 by compression tests. Bioink precursor mixed with MDA-MB-231 cells was printed into 4-layers lattice structure in 50 mM calcium contained agarose supporting gel by Regen HU printer[2]. The breast tumour 3D model was cultured up to 5 days and measured the cell viability.

RESULTS:OA were successfully functionalized considering an excess of aldehydes (37 mM) against available primary amines (4 mM).The selected hydrogel formulation (2%wt.OA(5%OD)-5%wt.gelatin) was then crosslink with various Ca^{2+} concentration (0.1 M to 0.5 M) to meet the stiffness criteria 2-18 kPa. We observed good cell viability (>80%) in this group of hydrogel up to day 5. Bioinks containing cancer cells were successfully printed showing >80% cell viability.

DISCUSSION & CONCLUSIONS:We have shown that OA-gelatin-calcium hydrogels are biocompatible, controlled in composition and stiffness and printable. This enables to model different stages of breast tumour progression in vitro, hence evaluate biomarkers expression, or to test treatment efficacy.

Acknowledgements:Chen Zhao would like to thank Dr. Samuel Moxon for 3D printing.

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Keywords: Disease models, 3D printing and bioprinting

Enzyme-mediated injectable poly(amino acid)s based hydrogels modified with RGD and GFOGER peptides for cartilage tissue engineering

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INTRODUCTION:The in situ formed biomimetic hydrogels represent a very attractive class of modern biomaterial scaffolds.Herein we present development of the synthetic, enzymatically degradable poly(amino acid)s based injectable hydrogel affording controlled immobilization of cell adhesion peptides RGD and GFOGER to encapsulate mesenchymal stem cells (MSCs), and evaluate the potential of this system for cartilage tissue engineering.

METHODS:The enzymatically degradable copolymer of poly(N5-2-hydroxypropyl-L-glutamine)-bearing tyramide units ((P2HPG-Tyr) was synthesized as the gel precursors from poly(γ-benzyl-L-glutamate) (PBLG) [1]. The hydrogels were formed in situ using dual syringe by horseradish peroxidase (HRP)/H2O2-mediated crosslinked reaction. The effect of various nH2O2/nTYR and nTYR/nHRP ratios on the time of gelation, gel yields, swelling, and viscoelastic properties were investigated. The biomimetic azido-(PEG)6RGDSGGGY-NH2 and azido-(PEG)6GFOGERGGGY-NH2 peptide ligands were covalently binding to the gel precursors using click chemistry in defined concentration.
For cell studies, hMSCs were encapsulated at a density of 3x106 cells/mL and cultured for up to 2 weeks in chondrogenic differentiation media. Live/dead staining was used to evaluate cell attachment and viability after 3, 7 and 14 days. Real-time PCR was used to evaluate the expression of chondrogenic markers (aggrecan, SOX-9, collagen I/II/IX).

RESULTS:The P2HPG-Tyr was modified using click chemistry with cell adhesion peptides RGD and GFOGER. The concentration of peptides in resulting hydrogels was range 0.5 – 5 mM.
The hydrogels were prepared with different stiffnesses (G' = 1.2 kPa and G' = 3.5 kPa). We observed good viability, adhesion, and proliferation of hMSCs after encapsulated by the injection process to the hydrogel. We evaluated the spreading of cells and the formation of the cellular protrusion in the 3D matrix depending on the RGD/GFOGER content and gel stiffness. The hMSCs adhered best to the hard hydrogel with the concentration of RGD or GFOGER higher than 2 mM. After nine-day of incubation in the chondrogenic medium the chondrogenic induction, expressions of collagen II proteins and chondrocyte-specific genes (SOX9, aggrecan and collagen II) were detected. The higher concentration of RGD/GFOGER peptides led to a less spreading area and a higher chondrogenic differentiation extent.

DISCUSSION & CONCLUSIONS:This study exhibited injectability and rapid P2HPG-tyr/RGD/GFOGER gel formation, as well as mechanical stability of hydrogel, and cell ingrowth. The P2HPG-tyr/RGD/GFOGER hydrogels demonstrated attractive properties for application in cartilage tissue engineering.

ACKNOWLEDGEMENTS:This work was supported by the Czech Science Foundation (No. 18-03224S).

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Keywords: Polymers - natural / synthetic / responsive, Stem cells – general

Characterization of fibrin scaffold structural properties using non-linear Optical Microscopy and Transient Grating

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INTRODUCTION:Fibrin is the main structural protein in blood clots: upon vascular injury, fibrinogen polymerizes forming a fibrous hydrogel able to withstand the forces exerted by the blood flow and embedded cells. Fibrin hydrogels have been extensively used as scaffolds for soft tissue regeneration.[1,2] Fibrin hydrogel mechanical properties can be varied as a function of fibrinogen concentration and thrombin content. Previous studies have analysed the mechanical, viscoelastic and structural properties of fibrin hydrogels as a function of fibrinogen concentration using physical contact between the probe and the material.[3] The aim of this study was to use non-linear optical microscopy techniques for fibrin hydrogel structural characterization.

METHODS:Fibrin hydrogels were prepared using different fibrinogen concentrations (5 mg/ml to 50 mg/ml) and human cardiac fibroblasts were embedded into hydrogels. Non-cellularised hydrogels were also prepared.
Transient Grating (TG) and two-photon excitation fluorescence (TPEF) microscopy were used to characterize the stiffness and structural conformation properties of cellularised and not-cellularised hydrogels. TG was performed by excitation of samples using two laser pulses. Light matter interaction generates a local transient diffraction grating and a standing pressure wave. Intensity modulations of the diffracted light from a third laser beam are monitored and are related to the propagation of the acoustic wave in the medium, its damping and thermal relaxation, allowing the measurement of sample bulk modulus.
The fibrous structure of fibrin hydrogel was characterized by TPEF microscopy, using an amount of fluorescently labelled fibrinogen before thrombin polymerization.

RESULTS:TPE imaging allowed to characterize the mesh size and structural spatial correlation, while TG gave information on bulk elastic modulus. The speed of sound in the medium and, hence, the bulk elastic modulus, increased as fibrinogen concentration increased, while the average mesh size decreased as a function of concentration, in agreement with previous findings and with TG results.

DISCUSSION & CONCLUSIONS:Elastic modulus of cellularised and non-cellularised fibrin hydrogels was measured by a combined approach using nonlinear optical techniques. The proposed methodology represents a tool to characterize scaffold remodelling by embedded cells.

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

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Keywords: Biomaterials, Decellularised matrices

A 3D immunomodulatory platform using liquefied and multilayered capsules

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INTRODUCTION:Liquefied and Multilayered Capsules (LMC) avoid the use of conventional scaffolds with fixed geometries and open surgery implantations.[1] LMC are composed by (i) a layer-by-layer membrane that ensures the diffusion of essential molecules, and (ii) a liquefied alginate core containing cells and microparticles. While within the liquefied core the encapsulated cells can move freely, microparticles provide the required adhesion domains. We aim to construct an engineered indirect co-culture system with macrophages (Mϕ) and stem cells. Accumulating evidences indicate that only an efficient and timely switch from pro-inflammatory to regenerative Mϕ phenotype results in a proper tissue remodelling process. Therefore, LMC ending with different types of polyelectrolytes are placed on top of 2D cultured Mϕ. The effect that encapsulated adipose-derived stromal cells (ASCs) have on the biological performance of Mϕ is also evaluated.

METHODS:Microgels are obtained crosslinking alginate containing cells and polycaprolactone microparticles (μPCL) in a CaCl2 solution. Then, layer-by-layer is performed using poly(L-lysine) (PLL), alginate (ALG), and chitosan (CHT) as polyelectrolytes to build the multilayered membrane. Three different encapsulation systems are developed, each one ending with a different polyelectrolyte. Ultimately, the liquefied core is obtained by EDTA treatment. LMC with or without encapsulated ASCs, and with variable last layers, are added on top of 2D cultured Mϕ. A 2D culture of Mϕ without LMC was used (control).

RESULTS:LMC without cells were cultured on top of Mϕ for 7 days. IL-6/IL-10 ratio significantly decreased over time, with a pronounced consistency for CHT. The gene expression of the pro-healing markers CD163 and CCL13 was increased for PLL and CHT, regardless the significant enhancement of inflammatory genes (CCL20/CXCL10) for PLL-ending LMC. Results show that ASCs significantly increased the release of IL-10 by Mϕ. Remarkably, the switch of pro-inflammatory to remodeling Mϕ phenotype was also confirmed by the enhancement of pro-healing markers and decrease of inflammatory markers.

DISCUSSION & CONCLUSIONS:Our data allow to select which last layer should LMC present to maximize the pro-healing response after implantation. The indirect co-culture shows the distinct immunomodulatory ability of ASCs when encapsulated in LMC. With the immune system as a crucial component influencing tissue regenerative process, we believe that the present study give important insights to ameliorate the outcomes of LMC as medical implants for tissue engineering therapies.

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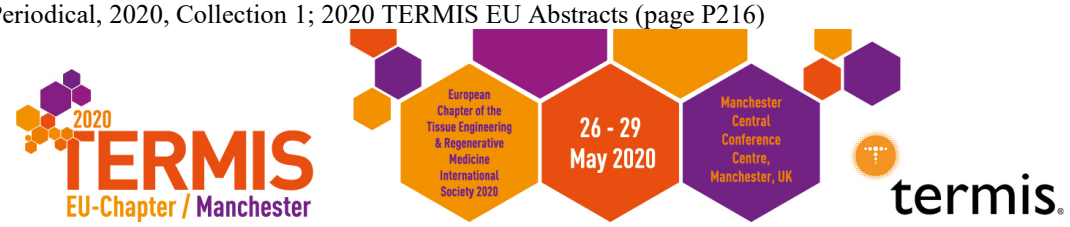
Keywords: Stem cell niche, Microenvironment and niche engineering



Evaluation of Immunological compatibility of marine-derived collagen and gelatin

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INTRODUCTION:Effective replacement and/or regeneration of damaged tissue or organs with a well-designed and appropriate construct is the leading goal of Tissue Engineering and Regenerative Medicine (TERM). Several strategies, such as the use of combinatorial biomaterials, cells and biochemical signals, but also the biomaterial per se, can be used to stimulate and induce the regeneration of host tissue itself[1]. The successfulness of this relies on the host response to the biomaterial, namely, the reaction of immune cells to implant.
Marine-derived collagens and gelatines are promising biopolymers to be used as building blocks for the development of biomaterials in the field of TERM. The absence of any religious constraints and risks associated to zoonosis (in opposition to bovine collagen and the related bovine spongiform encephalopathy, BSE) have demonstrated to be important advantages. However, there is a lack of information in literature about immunological impact of those materials, existing only a few reports using marine collagen from squid[2], tilapia[3], jellyfish[4] and carp[5].
Considering all that, this work represents, to our best knowledge, the first specific study of immunological response of different marine origin collagens and gelatines envisaged to be used as building-blocks for biomaterials.

METHODS:By-products from Galician and Portuguese fishing industries, particularly shark and codfish skins were used as raw materials to isolate collagen and gelatin. Bone Marrow Derived Macrophages (BMDM) isolated from C57Bl.6 mice were stimulated with the extracted type I collagen and gelatine for different time points and both cells and supernatant were collected for further analysis by qRT-PCR and ELISA.
RESULTS:Biomaterials endotoxin levels were assessed and proved to be lower. Results obtained from gene expression and cytokine quantification showed an activation of pro-inflammatory cytokines at early stages, suggesting that the studied materials induce an inflammation state in early moments of stimulation. However, the expression of IL-10 cytokine combined with the higher expression of Arg1 and lower expression of NOS2 suggest a translation to an anti-inflammatory scenario at later stage.
DISCUSSION & CONCLUSIONS:Gene expression and cytokine profile suggest that the tested collagenous materials induce a M2-like phenotype (recognized as anti-inflammatory and pro-regenerative) of BMDM, indicating that those biopolymers can create an advantageous cell-microenvironment for tissue regeneration.
ACKNOWLEDGEMENTS:European Union Transborder Cooperation Program Interreg España-Portugal 2014-2020 (POCTEP): projects0245_IBEROS_1_E and 0302_CVMAR_I_1_P and FCT Doctoral Program Do*Mar (ALA scholarship:PD/BD/127995/2016).
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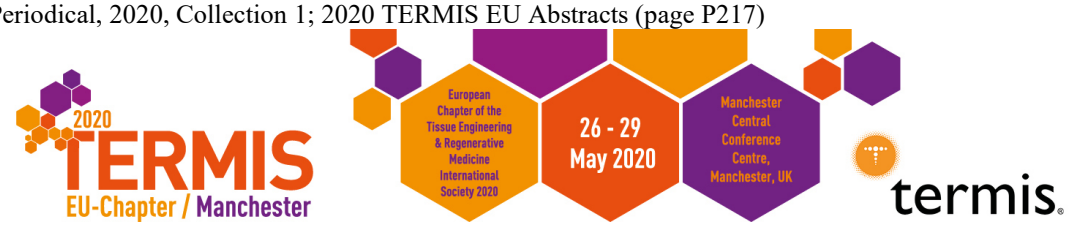
Keywords: Biomaterials, Polymers - natural / synthetic / responsive



Immune prints of devitalized cartilage grafts correlate with outcome of bone formation in immuno-deficient and competent mice

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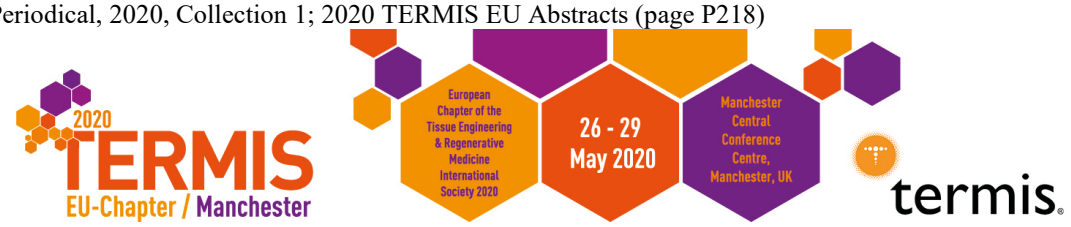
INTRODUCTION:Bone formation is a complex and dynamic process which occurs during development and fracture healing. Understanding the associated cascade of cellular events would help the design of grafting material capable of inducing effective regeneration. Typically, immunodeficient (ID) mice are considered standard for assessing the regenerative capacity of bone grafts. However, this model is of lower relevance towards preclinical validation because of the deficiency in immune system. Here, we investigate the impact of the immune cells on the remodeling of our graft into bone, in both immunocompetent (IC) and ID mouse models.

METHODS:Our graft consists of in vitro engineered cartilage tissue produced by human mesenchymal stromal cell (hMSCs) lines. After cartilage formation, the tissue is subsequently devitalized by apoptosis induction, thus resulting in a cell-free graft aiming at instructing bone formation by endochondral ossification. The devitalized cartilage is implanted subcutaneously in the back of IC and ID animals for a maximum of 12 weeks. The early recruitment of immune cells (dendritic cells, monocytes, macrophages, natural killer, T and B cells) was assessed quantitatively by flow cytometry at 3, 7 and 10 days post-implantation. The formation of cartilage and bone tissue was evaluated using histochemistry, immunofluorescence and micro computed tomography (µCT).

RESULTS:We demonstrated the reproducible engineering of devitalized cartilage by exploitation of hMSCs line. Following in vivo implantation, a complete remodeling into bone was achieved in ID, whereas only minor calcification was observed in implants retrieved from IC mice. We further established the “immune prints” of our graft in both ID and IC settings, by compiling the differential innate and acquired immune cell recruitment at day 3, 7 and 10. This allowed the identification of a temporal divergence in the recruitment of dendritic cells and macrophages. As early as day 3, a more effective polarization of M2 macrophages was detected in ID animals.

DISCUSSION & CONCLUSIONS:Our study illustrates the significance of the initial immune response during bone formation, and the performance gap between ID and IC models. In ID, we observed a very effective bone formation correlated with an early M2 macrophages recruitment. Those have been described as active promoter of angiogenesis, tissue repair, and our results further support their involvement in bone remodeling. Ultimately, compiling such immune prints may not only be essential for assessing bone grafts immunogenicity, but also towards tuning their composition to activate/inactivate key immune pathways and ensure effective bone formation in IC setting.

Keywords: In vivo and animal models,



A Mathematical Model of Vascularisation after Peripheral Nerve Injury to Inform Seeded Cell Distributions in Engineered Repair Constructs

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INTRODUCTION:Around 5% of traumas result in Peripheral Nerve Injuries (PNI). More than 1M people per year are affected in Europe and the USA [1], and healthcare costs are estimated to exceed £1Bn per year in the USA [2]. Paralysis and loss of sensation are hallmarks of severe PNIs, which can lead to lifelong pain and loss of autonomy for patients. Current treatments for large-gap PNIs use autografts to bridge between nerve stumps, inducing donor-site morbidity, often yelding underwhelming functional recovery [3].
Engineered Neural Tissue (EngNT) [4], comprising anisotropic cellular hydrogels reproducing properties of nerve tissue, is being developed to address these issues. EngNT constructs provide supportive microenvironments to promote neurite and vessel growth between nerve stumps, and enable careful spatial seeding of therapeutic cells to facilitate this.
Here, we consider vascularisation of the injury site, which is essential to oxygen and nutrients delivery to the cell population. Seeded cells, under hypoxic conditions, release vascular endothelial growth factors (VEGF); diffusive gradients of VEGF act as chemical cues for the migration of endothelial cells and formation of new microvessels [5]. This raises questions that are challenging to answer using experiments alone, for example what seeded cell densites or distributions will facilitate vascularisation and long-term cell survival?

METHODS:To help answering these questions we propose to expand the mathematical model reported in [6], which has been parameterised against in vitro data. This model comprises a system of coupled, non-linear, partial differential equations overlaid with a discrete model for endothelial sprout growth, reproducing, in silico, the spatio-temporal dynamics between blood flow, angiogenic processes, oxygen delivery, VEGF production and seeded cell viability during nerve injury repair.

RESULTS:Simulations were performed for a range of cell seeding densities and distributions, highlighting the impact on the growth of vascular networks and cell survival. Results predict the spatio-temporal distribution of oxygen and VEGF throughout the repair construct, and the complex interplay between these distributions, the cell population, and the vasculature.

DISCUSSION & CONCLUSIONS:This computational-experimental approach indicates new avenues for cell seeding strategies that may accelerate vascularisation of a repair construct and improve cell survival. In this way, mathematical modelling can be used to explore a variety of parameters and help inform future experimental repair strategies.

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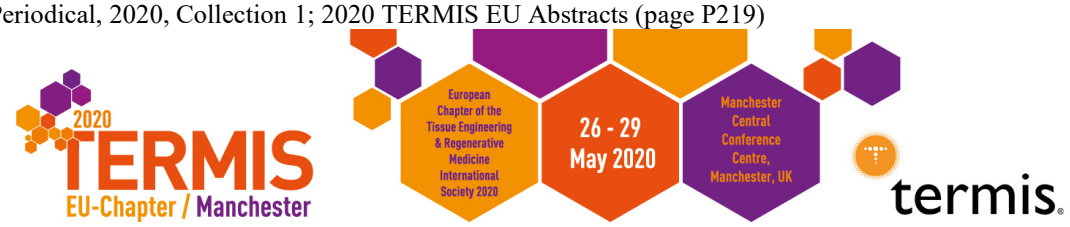
Keywords: Nervous system (brain-central-peripheral / disorders), Trauma / surgery and rehabilitation



Optimisation of nozzle design and process parameters for extrusion 3D bioprinting using in silico modelling

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INTRODUCTION:3D-bioprinting using hydrogel-based materials is one of the flourishing technologies in tissue-engineering. The scalability or wide application of this booming technology is often hindered by poor printability and cell survival. Trial and error in vitro approach is widely followed for process optimisation but requires more resource and time. To overcome these, in silico modelling of specific parts of the bioprinting process can be an effective approach. One of the most important factors dictating the success of 3D-bioprinting is shear stress. In our model, we correlate maximum shear stress (MSS) generated in the nozzle during printing, with cell-survivability. Based on this, appropriate parameter choices for print-nozzle is provided, with commonly utilised shear-thinning hydrogels in 3D-bioprinting.

METHODS:Three widely used natural and synthetic shear-thinning hydrogel materials (alginate, alginate-gelatine and pluronic-F127) were used to simulate flow in two nozzle configurations (blunted, conical). All the design parameters were varied in the range relevant for practical application, using space-filling Latin hypercube sampling (LHS). Different combinations of these design parameters were subjected to computational fluid dynamics (CFD) simulations to obtain flow profile and shear stress responses in each nozzle, at different printing pressure levels. The outcome of ~1600 different combination is fitted into the Gaussian process (GP, a machine learning method) to obtain individual design parameter response on MSS.

RESULTS:The relative influence of each variable on the maximum shear stress can be estimated in the range in which the value of the variable is varied, from GP. The importance of each parameter in nozzle design and relationship with the magnitude of shear stress is also obtained.

DISCUSSION & CONCLUSIONS:We found that the lower-nozzle length and nozzle-exit radius are the most important parameters for blunted nozzles whereas for the conical nozzle, middle and exit radii are crucial factors in dictating MSS. Material property (power-law index) was also shown to have important effects such as highly shear-thinning materials lead to lower MSS. The current approach is able to eliminate non-influential parameters and provide quantitative description for the important ones, from broad range screening, which would be almost impossible through conventional in vitro methods. In summary, the efficacy of in silico modelling is demonstrated, as a feasible approach to overcome costly experimental trial and error, to optimise the printer parameters, and to develop new bio-printable materials.

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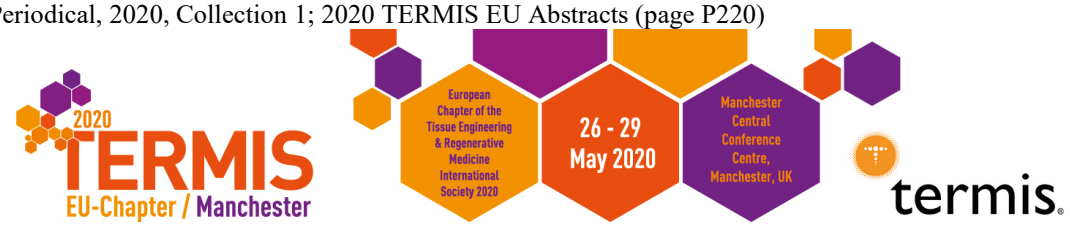
Keywords: 3D printing and bioprinting, Additive manufacturing



Multicomponent Hydrogels for the Formation of Vascularized Bone-like Constructs in vitro

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INTRODUCTION:The native extracellular matrix (ECM) is a complex gel-like system with a broad range of structural features and biomolecular signals. Hydrogel platforms that can recapitulate the complexity and signaling properties of this ECM would have enormous impact in fields ranging from tissue engineering to drug discovery. Here, we report on the design, synthesis, and validation of a microporous and nanofibrous hydrogel exhibiting multiple bioactive epitopes designed to recreate key features of the bone ECM.

METHODS:The material platform integrates self-assembly with orthogonal enzymatic cross-linking to create a supramolecular environment comprising hyaluronic acid modified with tyramine (HA-Tyr) and peptides amphiphiles (PAs) designed to promote cell adhesion (PA1), osteogenesis (PA2), and angiogenesis (PA3).

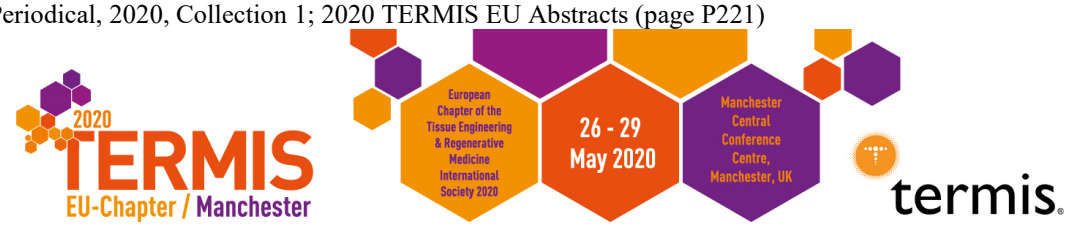
RESULTS:Through individual and co-cultures of human adipose derived mesenchymal stem cells (hAMSCs) and human umbilical vascular endothelial cells (HUVECs), we confirmed the capacity of the HA-Tyr/PA1/PA2/PA3 hydrogel to promote cell adhesion as well as osteogenic and angiogenic differentiation in both 2D and 3D setups. Furthermore, using immunofluorescent staining and reverse transcription-quantitative polymerase chain reaction (RT-qPCR), we demonstrated co-differentiation and organization of hAMSCs and HUVECs into 3D aggregates resembling vascularized bone-like constructs.

DISCUSSION & CONCLUSIONS:Bioactive environments that can recreate key properties of the native ECM and induce multiple cell types are essential for the kind of cell differentiation and organization properties required in effective in vitro models. We have developed a multifunctional hydrogel designed to promote osteogenesis and angiogenesis by recreating key structural and signaling elements of the native bone environment. The system takes advantage of both non-covalent and covalent interactions and enables the incorporation of specific bioactive epitopes and ECM components within a nanofibrous and microporous architecture. We have demonstrated how the multicomponent HA-Tyr/PA1/PA2/PA3 hydrogel can promote hAMSC adhesion and osteoblastic differentiation in addition to inducing HUVECs to grow into vascular tubules. By co-culturing these cells, we verify the capacity of the hydrogel to generate bone-like constructs in vitro. The results demonstrate the potential of these hydrogels to serve as complex in vitro environments as well as potential in vivo materials for bone tissue engineering.

ACKNOWLEDGEMENTS:This work was financially supported by the Scientific and Technological Research Council of Turkey (TUBITAK) with the 1059B191601219 numbered project, the ERC Starting Grant (STROFUNSCAFF), AO Research Fund of the AO Foundation project number AOCMF-17-19M, and the UK Regenerative Medicine Platform (UKRMP2) Acellular Smart Materials

REFERENCES:N/A

Keywords: Biomaterials, Other



A multidisciplinary approach to improve cell transplantation therapy for Parkinson's disease

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INTRODUCTION:Parkinson's disease (PD), has been shown to affect more than 1% of the population over the age of 60, and due to population growth and an ageing population this is expected to increase with time. Cell replacement therapy, in particular using ventral midbrain dopaminergic progenitors differentiated from human pluripotent stem cells, has emerged as a promising strategy for modifying PD's progression. However, both pre-clinical and clinical evidence shows high rates of cell death after implantation, possibly due to the effect of mechanical and oxidative stresses during injection and the host immune response1. Embedding cells within a biomaterial can confer them protection and promote engraftment2. However, optimising parameters such as cell seeding density and spatial distribution can require extensive experimentation which hampers progress in this area. Here we propose a multidisciplinary approach to determine the optimal seeding density and distribution of therapeutic cells in a candidate biomaterial in order to promote transplanted cell survival.

METHODS:The methodology employed can be divided into three stages; firstly an appropriate biomaterial for cell encapsulation is identified. Secondly, targeted experiments are performed to further our understanding of the relationship between the cell seeding density, oxygen availability and cell viability. Thirdly and finally, a mathematical model is developed and implemented to capture the interactions between these variables within a realistic geometry and arrive at the optimal conditions, in order to ensure cellular survival.

RESULTS:Data indicate that the use of biomaterials could provide a supportive matrix for therapeutic cells, thereby enhancing their survival under different culture conditions. After the choice of a suitable biomaterial, the work will progress into a cycle of iteration between in vitro experiments and in silico optimization to identify promising strategies to take forward to further preclinical testing.

DISCUSSION & CONCLUSIONS:The methodology proposed here aims to become a standard in the field of tissue engineering by leveraging and integrating methods from mathematical modelling, cell biology, biomaterials science and bioengineering. The framework developed is flexible and can be easily adapted to other scenarios as future material and cell technology options evolve.

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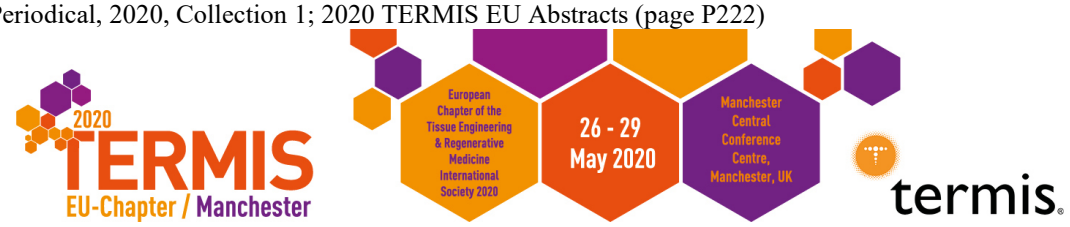
Keywords: In silico models,



A collagenous biofabricated "in vitro" model of pancreatic cancer to study importance of microenvironment on cell behavior and treatment design

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INTRODUCTION:With a survival rate of 9% (www.pancan.org), pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers worldwide, mainly due to its its high resistance to traditional therapeutic methods, which has been attributed to its complex tumour microenvironment (TME) containing various cells types and a complex extracellular matrix (ECM) [1]. This low survival rate emphasizes the need for a more in-depth study of the TME as well as the various therapeutic methods using more physiologically relevant models such as three-dimensional (3D) in vitro ones that better mimic the in vivo niche [2,3]. Herein the use of a novel biofabrication technique is reported to develop and characterize a 3D model of PDAC involving cellular aggregates and ECM protein as a rapid, high throughput system to assess the effects of various treatment methods.

METHODS:A previously developed biofabrication technique [4] was used to make in vitro models of PDAC using different cell lines (PANC-1 and BxPC-3) with precise control over construct sizes and compactness through variations in collagen concentrations and cell seeding density. Alamar Blue assay, immunofluorescence staining, and bright filed microscopy were carried out at specific time points to assess cell viability, construct size, and cell specific marker expressions.

RESULTS:Collagenous grafts with the same number of cells and different degrees of compaction were fabricated. Viable and proliferative cells were observed throughout the constructs for different sizes with more uniform distribution within aggregates of lower compactness. Diffusion limitation studies with DAPI suggested that the compactness of the aggregates had greater effect on cells' behavior rather than aggregate sizes. This contrasts with the popular belief that diffusion limitation is mostly affected by graft size.

DISCUSSION & CONCLUSIONS:Here, a new graft fabrication technique was used to develop a rapid in vitro model of PDAC with controlled size, compaction, and mechanical properties of the constructs using different PDAC cell lines. In contrast to current theory, the study highlights that construct compaction is the dominant factor for mass transfer limitations and not the aggregate size. This model could further be improved by establishing co- and tri-culture of cancer cells with other cell types including parenchymal and stromal cells to make the model more physiologically relevant.

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Keywords: Biofabrication, Cancer

Microfluidic environment as minimal functional unit for primary human osteoblast differentiation

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INTRODUCTION:Microengineered environments provide an in vitro strategy to explore the variability of individual patient response, since they prefer the use of primary cell sources representing the phenotype variability. In this study, we want to translate the findings of traditional macro-models on osteoblast differentiation to the microfluidic scale [1], taking advantage of the limited number of cells required by these systems. By culturing primary human osteoblasts in a 3D fibrous collagen matrix, we hypothesized that the bone on a chip technology creates a robust model to recapitulate osteoblast maturation towards osteocytes.

METHODS:Primary human osteoblasts were seeded in a type I collagen hydrogel and cultured up to 21 days, to establish the role of lower (2.5x10^5 cells/ml) and higher (1x10^6 cells/ml) cell density on their differentiation into osteocytes. Variation in cell morphology was assessed with a custom semi-automatic image analysis software to extract quantitative data on cell dendrite length. Cell proliferation (DNA content) and extracellular alkaline phosphatase (ALP) activity were measured to monitor cell proliferation and osteogenic activity over time. To evaluate protein synthesis, immunofluorescent staining of bone sialoprotein 2 (BSP2, osteoblast marker) and dental matrix protein 1 (DMP1, osteocyte marker) was performed.

RESULTS:The image analysis software quantified a significant dendrite growth of 1.01±0.25 mm/day for human osteoblasts cultured at high density. DNA content did not significantly change over time for the high cell density group, while extracellular ALP activity showed a significant upregulation by day 7 followed by a decreasing trend. Immunofluorescent staining for DMP1 confirmed the synthesis of the osteocytic marker. On the other hand, osteoblast cultured at low density did not increase dendrite length over time, increased the DNA content and had a lower ALP activity with no significant variation. Cells synthesized BSP2 over the whole period of culture while DMP1 staining was negative.

DISCUSSION & CONCLUSIONS:Overall, our data showed how the 3D microenvironment in our bone-on-a-chip regulated osteoblast-osteocyte differentiation, which depended on cell seeding density. Only the human osteoblasts seeded at higher density underwent the changes in cell morphology, proliferation, osteogenic activity and protein production that are specific of their differentiation into osteocytes. From a clinical perspective, the low volumes of culture in such a system enable highly reproducible patient-specific devices to estimate the regenerative potential of the patient bone cells.

Acknowledgements:The CuraBone project received funding from ITN-MSCA, grant agreement No. 722535.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Personalised medicine

Next generation of tissue culture plates via micro and nano-surface engineering

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INTRODUCTION:Tissue culture plates are widely used due to their ease of use and compatibility with many biological assays. However the planar nature of the surfaces of these plates present a dramatic difference compared to any conditions encountered in real biological environments. There is an increasing need for a new culture format that better provides cues for cell cultures whilst retaining the ease and compatibility of standard TCPs for cellular analyses.
Here we propose the use of a novel method to directly implement engineered surface micro-/nano-environments on standard tissue culture plates (TCP). These enable the use of standard rapid screening techniques to analyse libraries of topographies over a well-known and understood material.

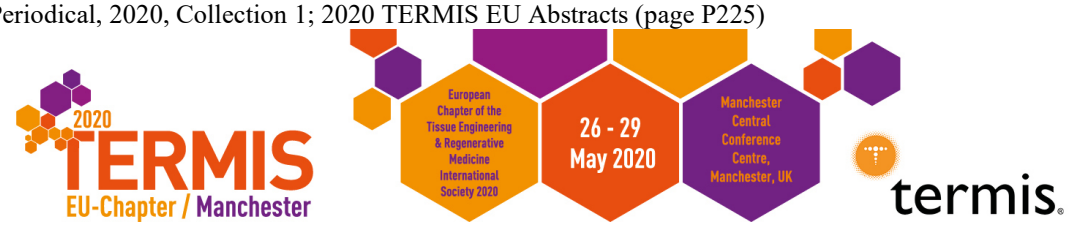
METHODS:Surface engineered TCPs were modified by using hot-embossing to present a large number of topographies and geometries on their surface for screening purposes. Fabricated surfaces were characterized using AFM, SEM, XPS and contact angle analysis. Prior to cell seeding the surfaces were plasma activated and a range of cells, including mesenchymal stromal cells, keratinocytes, fibroblasts, endothelial and myoblasts, were cultured for 72 hr. Morphological changes and cell type-specific functional responses were quantified via immunofluorescence and image analysis.

RESULTS:Surface analysis proves that there is not significant difference between un-modified and processed TCP in terms of surface chemistry or hydrophilicity. No difference in cell viability, adhesion or spreading capabilities was observed between the unprocessed TCP and flat surface-engineered control surfaces. Conversely, samples exhibiting micro- or nano-features induced significant changes to the cellular morphology, in a manner specific to the particular pairing of geometry and cell type. Finally, functional assessment showed distinct changes to cell-specific functional marker levels and localisation in a pattern-dependent manner.

DISCUSSION & CONCLUSIONS:The proposed technology has been adapted to be compatible for off the shelf TCP (proven from 6 to 96 wells). Our data on cell analysis highlights the utility of this system to rapidly screen for architectures that provide the optimum phenotype for any particular cell, as well as providing proof-of-concept that engineered TCP can be used to modulate cell activity in culture. Overall it demonstrates the versatility of this method and future prospects to unlock the potential of in-vitro cultures beyond the use of conventional TCPs.

Acknowledgements:Financial support was received from the Australian Research Council (DP190100129), Monash University and CSIRO Interdisciplinary Research schemes. This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF).

Keywords: Microenvironment and niche engineering, Interfaces – engineered



Three-dimensional engineered capsules as bone marrow microniches for regenerative applications

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INTRODUCTION:The development of an in vitro human bone marrow (BM) niche is essential to understand the crosstalk between the different key players in the bone microenvironment. Stem cell-based bone tissue engineering has emerged as a promising approach for regenerating critical bone defects. However, conventional 3D prototypes have failed in the establishment of a dynamic model able to mimic the complexity of the bone marrow environment in vitro. The aim of this work was to develop multifunctional liquefied capsules mimicking the BM microenvironment. For that, mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs) and human umbilical vein endothelial cells (HUVECs) were co-encapsulated. Interestingly, human umbilical cord was the common source to isolate all the multiphenotypic cells herein used.

METHODS:Multilayered and liquefied alginate capsules co-encapsulating cells and surface-modified polycaprolactone microparticles (µPCL) were produced as previously described (Correia et al. 2013; Correia et al. 2019). Two types of capsules were produced, namely tri-culture capsules with MSCs, HSCs and HUVEC, or co-culture capsules with MSCs and HUVECs. Different cell ratios were tested. Capsules were maintained in basal or in osteogenic differentiation media, and analyzed at 3, 7 and 10 days.

RESULTS:The metabolic activity and cell proliferation increased in tri-culture capsules compared to co-culture capsules. This evidences the superior biological outcome resulting from the direct crosstalk of the three phenotypic cells within the privileged environment of capsules. We also observed the presence of osteoblastic bone formation markers such as alkaline phosphatase, osteopontin and osteocalcin in both type of capsules, but more significant in tri-culture capsules cultured in osteogenic differentiation medium.

DISCUSSION & CONCLUSIONS:Notably, our results demonstrate that in vitro microniches were successfully obtained inside the capsules with appropriate diffusion of essential molecules that support the maintenance and proliferation of HSCs, their in vitro stemness characteristics, and the capacity of MSCs to differentiate towards the osteoblastic lineage even in the absence of supplemental osteogenic differentiation factors. Our multifunctional liquefied capsules mimicking the BM microenvironment provide an advanced platform of fundamental importance for new bone regeneration applications.

Acknowledgements:The authors acknowledge the financial support by the FCT for project CIRCUS (PTDC/BTM-MAT/31064/2017), and the European Research Council for project ATLAS (ERC-2014-ADG-669858).

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Keywords: Cell therapy, Microenvironment and niche engineering

Mimicking the effects of articular joint inflammation in a multi-tissue microfluidic osteochondral model

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INTRODUCTION:Cartilage degradation along with alteration in subchondral bone remodeling and angiogenesis make osteoarthritis (OA) a whole joint disease [1]. In this context, the development of advanced in vitro models able to mimic complex anatomical districts is crucial to study the disease and test potential therapeutic agents. Here, we modeled the osteochondral interface in a microfluidic platform with the aim of investigating the cartilage-bone crosstalk and the expression of factors involved in cartilage degeneration, bone remodeling and angiogenesis in healthy and OA-like conditions.

METHODS:The microfluidic chip comprises the bone and the cartilage compartments in direct contact. The cartilage compartment is composed of human articular chondrocytes embedded in fibrin hydrogel. The bone compartment consists of osteoblasts, osteoclasts, and endothelial cells, embedded in fibrin hydrogel enriched with calcium phosphate nanoparticles to mimic the mineralized part of the bone [2]. After 4 days, the chips were treated with interleukin-1β (IL-1β) to induce inflammation. On day 7, the samples were analyzed by immunofluorescence to assess markers related to cartilage degradation, osteoclastogenesis, and angiogenesis. The microvascular network formed within the model was also analyzed by FIJI software.

RESULTS:Matrix metalloproteinases were expressed by osteoclasts both in control and IL-1β-treated samples, being slightly upregulated in the presence of IL-1β. Differently, chondrocytes showed the expression of matrix metalloproteinases only when treated with IL-1β. Osteoclastogenesis was also upregulated in IL-1β-treated samples. Regarding angiogenesis, vascular endothelial growth factor was highly expressed only by endothelial cells in control samples, whereas its expression was strongly upregulated when the samples were treated with IL-1β. Additionally, we found that the morphology of the microvascular network changed between control and IL-1β treated samples, with the latter being characterized by thinner and more elongated vessels.

DISCUSSION & CONCLUSIONS:We developed the first microfluidic osteochondral model including cartilage and vascularized bone. We demonstrated that inflammatory conditions stimulate chondrocytes to express matrix degradation factors, regulate osteoclastogenesis, and involve osteoclasts in angiogenesis augmentation. This complex model represents a step forward in the study of disease-related mechanisms involving the crosstalk of bone and cartilage, which could be applied in the study of OA as well as of other multifactorial joint diseases.

ACKNOWLEDGEMENTS:This project was funded by the Italian Ministry of Health (Ricerca Corrente).

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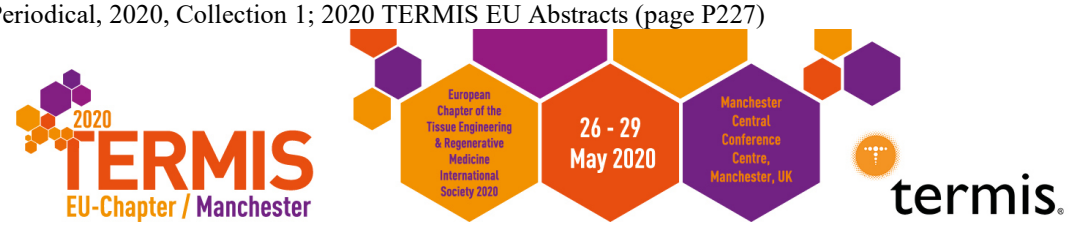
Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Cartilage / joint and arthritic conditions



Can HepaRG self-assembled spheroids meet the clinical needs for extracorporeal liver supply?

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INTRODUCTION:It is recognized that culturing cells in spheroids is advantageous as they better reproduce the three-dimensional physiological microenvironment. This approach can be exploited in bioartificial liver (BAL) applications, where obtaining a functional hepatic biomass is the major challenge. In this study, we encapsulated HepaRG cells (precursors of hepatocyte-like cells) in alginate 1.5% without pre-forming spheroids and analysed those activities required for a BAL therapy in the treatment of acute liver failure (ALF).

METHODS:HepaRG cells from Biopredic (Rennes, France) were encapsulated in alginate beads by coaxial-airflow extrusion method. The encapsulated cells were transferred to culture dishes and maintained for 14 days in continuous orbital shaking(1). Cell differentiation was studied during 14 days of culture. On days 7 and 14 post-encapsulation, cell performance was tested by metabolic and xenobiotic tests, which include respectively albumin synthesis and ICG, EROD and BROD assay. In parallel, ammonia and lactate detoxification rates were studied on a pathological plasma model.

RESULTS:Cells remain highly viable over 14 days of culture. They are capable to self-rearrange in spheroids, differentiate and display a wide range of hepatic features. The cells respond to the clinical needs of BAL therapy(2) being capable to produce albumin (rate of 0.64±0.21 µg/h/106), detoxify ammonia (118.75±23.06 nmol/h/106), and lactate (166.75±23.66 nmol/h/106), and to express the enzymes involved in the xenobiotic machinery (such as CYP 1A1/2), with highest activity on day 14.

DISCUSSION & CONCLUSIONS:Starting from a given hepatic biomass, we analysed cell differentiation and metabolic performance for further use in fluidized-bed-BAL(1). We observed that cells rearranged as aggregates into the beads, with dimensions within a range (maximum 120 µm) where mass transfer is not affected(3), and adequately differentiated over time. At day 14 post-encapsulation, cells displayed a large set of hepatic features necessary for the treatment of ALF. Waiting for fully mature hepatic cells issued from pluripotent stem cells, this HepaRG based bioconstruct demonstrated its potential for further use on extracorporeal treatment of ALF.

ACKNOWLEDGEMENTS:The research project is funded by ANR16-RHUS-0005 in the framework of the iLite programme The HepaRG cell line, media and supplements used for this investigation were supported by Biopredic International, as part of this programme.

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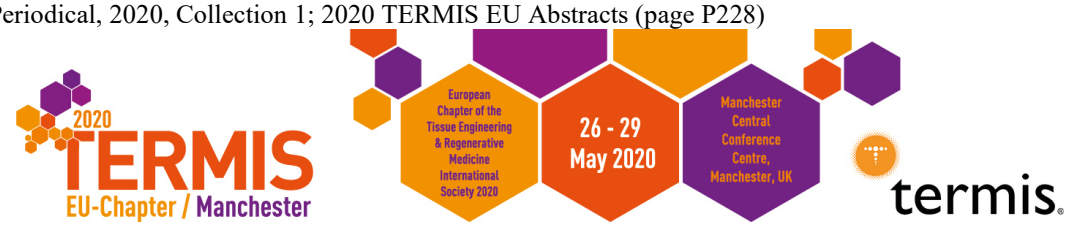
Keywords: In vitro microenvironments, Bioreactors



Human Adipose Stem/Stromal Cell Spheroids as a Model of Osteogenesis

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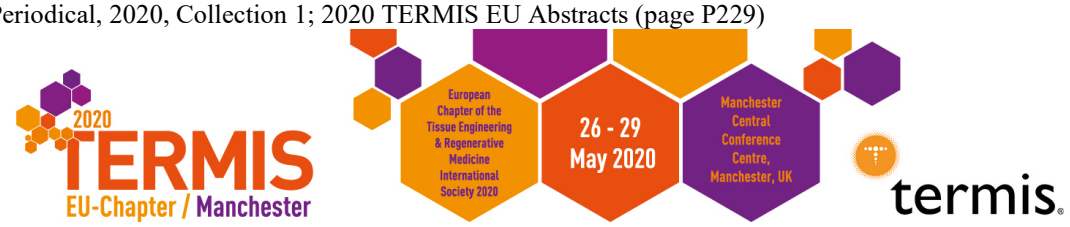
INTRODUCTION:New bone formation may be desirable in a variety of clinical settings, such as osteoporosis and non-union fractures. Adipose derived stem/stromal cells (ASCs) are considered a promising cell source for bone regeneration through tissue engineering techniques. A new approach in this fied corresponds to the use of stem-cell spheroids as modular units to engineer biological tissues in vitro.

METHODS:ASCs were isolated by mechanical dissociation from human lipoaspirate samples obtained according to the local research ethics committee. Initially, induction to osteogenic differentiation was performed in ASCs spheroids produced from micro molded agarose hydrogel. Spheroids were first maintained for two weeks in a chondrogenic medium composed of 10-8 mM Dexamethasone and 10 ng/mL TGF-β3. Then, the medium was exchanged for an osteogenic one composed of 10 mM β-glycerophosphate and 10-7 mM Dexamethasone for additional three weeks.

RESULTS:At week 2, induced spheroids presented upregulation of collagen type X ($p < 0.001$) and MMP-13 ($p < 0.0001$) genes compared with non-induced spheroids. Secreted levels of IL-6 ($p < 0.0001$), IL-8 ($p < 0.0001$), IL-10 ($p < 0.0001$), VEGF ($p < 0.0001$) and RANTES ($p < 0.0001$) were the highest at week 2. Induced spheroids showed strong in situ staining for collagen type X and low staining for the anti-angiogenic protein TSP-1 at week 2. Positivity for collagen type I, osteocalcin, biglycan and tenascin C was found at week 5, as also the presence of calcium deposits. Young's modulus values of induced spheroids were greater than 100kPa at week 3 and more than 10 times higher than non-induced spheroids. When submitted to in vitro fusion process, the induced spheroid duplets do not present the same fusion kinetics of the non-induced in the beginning of the process. Non-induced spheroids started to fuse faster when compared to induced spheroids, however, after 24h, the fusion kinetics was the same for the both groups until the end of fusion. In addition, induced spheroids at weeks 2, 3 and 5 when arraged in quartets for fusion assay, presented morphologycal differences.

DISCUSSION & CONCLUSIONS:Therefore, in this work, it was possible to prove the functionality of ASCs spheroids induced for the osteogenic pathway by molecular, mechanical and secretory tests of soluble factors. Besides, it was possible to understand better the differences in fusion kinetics and morphology of non-induced and induced spheroids, a process that is mandatory in order to use spheroids as building blocks in 3D bioprinting for bone tissue engineering approaches.

Keywords: Bone and bone disorders (osteoporosis etc), In vitro microenvironments



Decellularised extracellular matrix from mesenchymal stromal cells permanently cultured under different O2 levels: morphology and functional activity

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INTRODUCTION:The decellularised extracellular matrix (dcECM) of native tissues or cultured cells are attractive as a source of biological scaffolds for tissue reconstruction. Mesenchymal stromal cells (MSCs) are key components of connective tissue and produce various amorphous fibrillar ECMs depending on specific tissue milieu. The O_2 levels are crucial for microenvironmental regulation of MSCs functions, ECM production and remodeling, in particular. Here we prepared and characterizes dcECMs from MSCs permanently cultured at different O_2: normoxia (20% O_2) and "physiological" hypoxia (5% O_2).

METHODS:Human adipose-derived MSCs were permanently cultured at 20% and 5% O_2. Dense monolayers were decellularised with 0.5% Triton-X100/20 mM NH_4OH/PBS. Scanning electron (SEM) and confocal microscopy were used to examine dcECM morphology. To test functional activity (adhesion and osteo-differentiation), dcECM from 20% O_2 (20-dcECM) or 5% O_2 (5-dcECM) were recellularised with MSCs at 20% O_2. Collagen I coating was used for comparison.

RESULTS:According to SEM dcECMs retained the structural peculiarities of ECMs in monolayered MSCs. Under 5% O_2 dcECMs were formed by dense network of thin intersecting fibers, while membrane-like structures without separation into individual fibrils were noted at 20% O_2. In recellularisation assay, the efficiency of MSCs adhesion was higher on 5-dcECMs. At early adhesion, dish-shaped MSCs were prevailed on collagen, most of MSCs on dcECMs had numerous pseudopodia. Together with the morphology and structure of actin filaments this could be considered as indicator of a softer structure of dcECMs than of collagen. Both types of dcECMs effectively supported the MSCs expansion. Spontaneous and induced osteodifferentiation of MSCs were more pronounced on dcECMs than on collagen. The spontaneous activity of alkaline phosphatase was 2 times lower on 5-dcECMs, this effect was canceled upon osteoinduction.

DISCUSSION & CONCLUSIONS:It was demonstrated the possibility to obtain ECMs with certain properties depending on O_2 levels. The differences in 20- and 5-dcECMs structures with recently detected shift in the transcriptoion of ECM-associated genes, confirmed the dynamics of the ECM structure. Higher activity of MSCs alkaline phosphatase at 20-dcECMs as compared with 5-dcECMs is corresponding to direct effects of O_2 levels on MSCs differentiation. This fact can be considered as evidence of "ECM-trained MSCs" phenomenon.
The data hold promise for further investigation of ECM regulation of MSCs functions and application of dcECMs as biocompatible coatings for scaffolds.

ACKNOWLEDGEMENTS:This work was supported in part by Programm #19 of Presidium of Russian Academy of Sciences.

Keywords: Multipotent (mesenchymal) stem cells, Decellularised matrices

Development of tumour microenvironment models for analysis and imaging by MALDI mass spectrometry

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INTRODUCTION:During the evolution of cancer, many factors are involved in the interaction between malignant and non-malignant cells, resulting in continuous changes in the tumour microenvironment (TME) [1]. Extracellular vesicles (EVs) released from different tumours, including colorectal cancer, have shown to be preferentially uptaken by residential cells in specific sites of metastasis, contributing to the creation of the pre-metastatic niche [2]. The aim of this work is to develop models suitable for the imaging of CRC invasion which can be then utilised to assess the effect of EVs on the TME.

METHODS:3D models based on the invasion of CRC cells (SW480) were compared, adapted from an organotypic model of CRC previously developed by our research group. HFFF2 (human foetal foreskin fibroblasts) were embedded in extracellular matrix (ECM)-based gels (Collagen type I, GelTrexTM) in order to reproduce the TME in three different formats. A) The gel was prepared in the inner part of Transwell(R) plates and SW480 applied to invade from above. B) The gel was cast in ultra-low attachment well plates with a layer of SW480 above. C) Spheroids of SW480 were left to grow in ultra-low attachment well plates, before casting the gel on top. Haematoxylin & Eosin (H&E) staining was performed on sections obtained from the models. Proteomic signatures of organotypic models were investigated by MALDI mass spectrometry.

RESULTS:H&E staining of 3D models prepared in Transwell(R) plates (A) was able to show a clear front of invasion of SW480 increasing over time. Invasion was also observed in model B, however the level of invasion was limited compared to Transwell(R) model. A cancer mass was easily observable in spheroid model (C), but it appeared disjointed from the fibroblast layer. Preliminary mass spectrometry data based on Peptide Mass Fingerprint (Mascot search) presented putative identification of intracellular proteins linked to cytoskeletal remodelling and ECM.

DISCUSSION & CONCLUSIONS:3D Transwell(R) organotypic models appeared to best reproduce the TME, showing an increasing invasive front into a fibroblastic stromal layer with histological staining. As such, further studies focusing on tumour-derived EVs treatment of the TME before the cancer invasion will be conducted. MALDI-mass spectrometry is suitable to measure changes in TME composition as it can detect cellular and extracellular components in these models.

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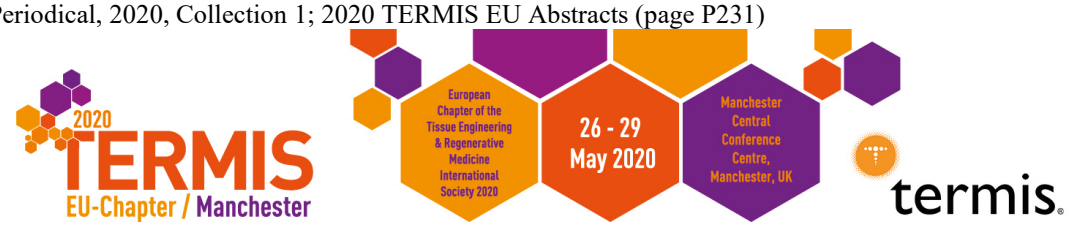
Keywords: Cancer, Disease models



A novel in-vivo model for the assessment of osseointegration of dental implants

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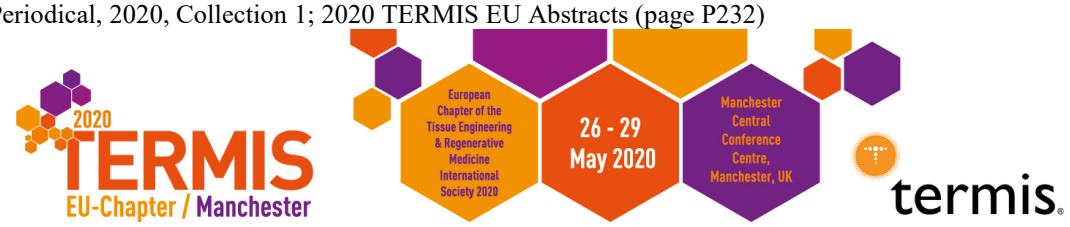
INTRODUCTION:Recently, there has been a remarkable expansion and innovation in implant manufacturing and implant tissue engineering. Satisfactory results in preclinical research are mandatory before translation into clinical trials. The selection of a suitable animal model is crucial to be able to answer a specific research question. Thus, the challenge to find a viable novel model to assess the osseointegration of new dental implants remains open.
Various approaches have been developed for the evaluation of osseointegration of dental implants in animal models, but the majority are not in the oral cavity. Studying the osseointegration in the mouth will provide insights into mechanisms and factors that affect the healing process. Extra-oral models using femur and tibia are not able to replicate the clinical situation where the implants are subjected to cyclical loading as well as exposed to the oral environment. Therefore, finding a model that meets these criteria is essential. To the best of our knowledge, the application of dental implants in the mouth of rabbits has not been previously described. Our aim is to demonstrate a new simple model to be utilized for dental implant research. The proposed model allows the evaluation of the osseointegration process both radiographically and histologically in addition to investigation of the impact of saliva and oral microorganisms on this process. Furthermore, due to the selected location, the implant will be subjected to indirect loading from the opposing lower incisors.

METHODS:The model was developed in male New Zealand white rabbits. The model allows for a randomised, controlled split-mouth design. In each rabbit, two implants; one experimental implant on one side, and one control implant on the other side were applied. Screw-shaped implants were used with a length of 8 mm and a diameter of 2 mm. The implants were inserted in the extraction sockets of the accessory incisors in the maxilla. This did not impact on the rabbit feeding postoperatively.

RESULTS:All rabbits tolerated the surgical procedures and recovered without complications. Our model provides a novel approach to study the osseointegration of dental implants in the oral cavity. Furthermore, this model can be tailored for the osteoporotic, immunocompromised and diabetic setting.

DISCUSSION & CONCLUSIONS:The described model for osseointegration of dental implants in the rabbit maxilla is safe, reproducible, standardised, requires minimal surgical skills and basic surgical instruments, and was relatively quick. Future studies can utilise the model for the evaluation of new dental implants prior to clinical application.

Keywords: Innovation, Translation and commercialisation (inc. clinical trials and regulatory approval)



In vivo evaluation of regenerative potential of connective tissue spheroids using bioreactors integrated with body

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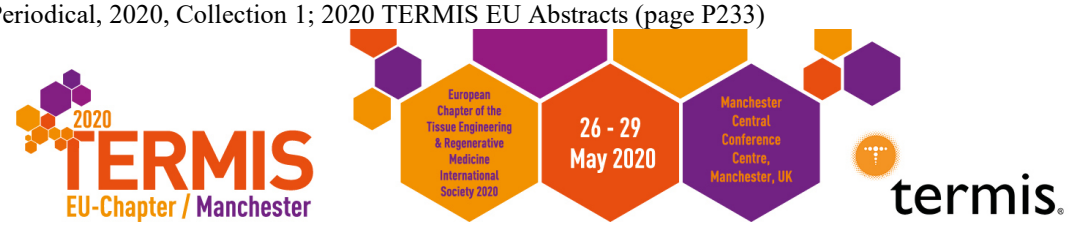
INTRODUCTION:Spheroids are densely packed cell aggregates which are used as building blocks in bioprinting and tissue engineering. In order to assess the regenerative potential of tissue spheroids we have for the first time used an in vivo bioreactor integrated into the body of an animal.

METHODS:The experiment has been carried out on white male laboratory rats (150–170 g). Cultures of papillary fibroblasts, rib perichondrium fibroblasts and femoral cortex fibroblasts have been used. Standard sized spheroids have been produced using 3D cultivation (MicroTissues Inc.®) of same-type cells. Spheroid viability has been analyzed using the LIVE/DEAD® method. Regenerative potential has been assessed using light and electron microscopy, morphometry, based on the speed of adhesion and spreading (changes in sphericity) of individual spheroids on the wound surface, the production volume of newly-formed structural elements and the kinetics of fusion between adherent spheroids.

RESULTS:Research has demonstrated that autologous spheroids of the same type (from cells of various connective tissue differentiations), when transplanted onto the wound on the back of a rat, regardless of the source of connective tissue cells, spread under the action of gravity and interact with the recipient surface in a similar way: they adhere to the tissues of the wound bed and spread gradually.

DISCUSSION & CONCLUSIONS:An objective assessment of the regenerative potential of spheroid cells in an in vivo animal model using quantitative and predictive assay is essential for improving the quality of spheroids and their production methods. In addition, experiments have shown the prospects of using in vivo bioreactors integrated into the body of a model animal, not only for the objective assessment of the regenerative potential of tissue spheroids, but also for choosing the optimal parameters of the local environment which supports organotypic regeneration and implantation of tissue-engineered constructs.

Keywords: In vivo and animal models, Wound healing



A tissue engineering approach to study bone metastases

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INTRODUCTION:Bone metastases are the most common cause of cancer-related pain and often lead to complication such as bone fractures and spinal cord injury. Even tumors such as breast cancer, that lead normally to a positive resolution, may present increased morbidity if they metastasize to bone. The development of models allowing the study of cause mechanisms and new therapies represent a great challenge in cancer research and animal models are essential for this purpose. There are already numerous models, however there is still no single standardized model.

METHODS:Our aim was to use a tissue engineering approach to create a mouse model and create a suitable environment for bone metastasis starting from human cancer cells deriving from breast cancer. [1] Human mesenchymal stromal cells (hMSCs) were seeded on a biocompatible and porous scaffold based on hydroxyapatite and β-tricalcium phosphate (MBCP+). The constructs were implanted in nude mice, to induce human bone growth. Once human ossicle was obtained in this "foreign" environment, human breast cancer cells were inoculated directly on site, at the implant site, or intravenously through the tail. We injected luciferase-expressing breast cancer cells and the colonization and metastases formation were followed by IVIS. Mice were sacrificed and the explants analysed by histology. Some explants were processed to recover the breast cancer cells that colonized the construct. They were selected because expressing zeocin resistance.

RESULTS:MDA-MB-231 metastatic breast cancer cells were injected intravenously in mice previously implanted with MBCP+ ± hBMSCs to obtain human bone. At first, cells colonized the lungs, but they were able to reach the human bone within two weeks. No colonization was observed in mouse skeleton. After death, explants were plated in presence of zeocin antibiotic for selection of tumoral cells only. Immunohistochemistry for luciferase showed that breast cancer cells were detectable only in blood vessels in the lungs, but we founded them inside the tissue in the bone explants.

DISCUSSION & CONCLUSIONS:The novelty of this work is the development of a method for recovery the metastatic cells. This allows to study the mechanism by which breast cancer metastasizes to the bone, creating a specific species environment and comparing the recovered cells with parental cells. It could be an excellent tool for testing new combinations of drugs and, by using the patient's cells directly, it could become a tool for the development of personalized therapies.

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Keywords: Cancer

In vivo assessment of 3-dimensional collagen matrices based on marine vs bovine origin collagen in a rabbit bone regeneration model

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INTRODUCTION:Collagen-apatite composite materials still stand out as particularly promising biomaterials for bone applications, since they offer attractive biological cues that encourage cell and tissue integration in vivo. Within the "collagen world", mammalian origin collagens, especially bovine, are still the most common ones. However, the risk of BSE transmission to humans limits its use. The growing demand for collagen-based therapeutic approaches and the limited sources for safer collagen have been driving the exploration of alternative sources. Among those, marine origin organisms have been studied, but despite many having been assessed, only a few in vivo evaluations of marine collagen biomaterials have been reported.

METHODS:Based on our recent published work (1), we performed the in vivo assessment of 3D matrices based on marine (mCol) or bovine (bCol) skin collagen combined with marine bioapatite (mBAp) from teeth of Prionace glauca or commercial hydroxyapatite (Ap), respectively. The stability of the 3 different formulations [mCol, mCol:mBAp and bCol:Ap (30%:70% w:w)] was increased by crosslinking with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) at 12.5 % (w:w). The produced structures were implanted in defects in condyles of rabbits and bone regeneration was assessed 12 weeks after implantation.

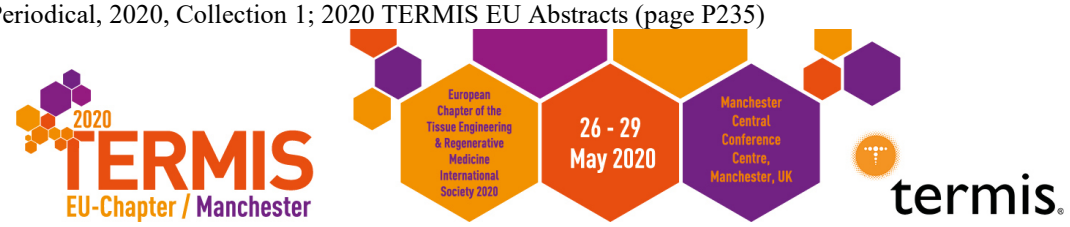
RESULTS:All scaffolds showed porous architecture with a pore size distribution between 10–500 µm, with higher compression moduli being exhibited by mCol:mBAp. Micro-CT at 12 weeks post-implantation showed that the scaffolds resorption was faster for mCol and bCol:Ap. There was new bone formation (bone volume/total volume) in all tested groups (bCol:Ap = 26 ± 10 %, mCol = 23 ± 8 % and mCol:mBAp = 20 ± 7.5 %), without significant statistical differences between them. A similar observation was made for bone mineral density: bCol:Ap = 0.37 ± 0.13 g/cm3, mCol = 0.37 ± 0.09 g/cm3 and mCol:mBAp = 0.32 ± 0.10 g/cm3.

DISCUSSION & CONCLUSIONS:Pore size and interconnectivity exhibited by the developed scaffolds enabled bone infiltration into them and new bone formation. It was hypothesized that the slower resorption rate of mCol:mBAp was related with its higher cohesiveness, revealed by increased mechanical properties. This lower biodegradation of marine origin composites might justify the apparently lower new bone formation and density on the respective group, despite statistical analysis revealing no significant differences. Histomorphological evaluation is underway to complement these findings. The proposed marine origin composites are herein disclosed as promising alternatives to mammalian and synthetic origin materials for bone tissue regeneration.

ACKNOWLEDGEMENTS:M-ERA-NET2/0022/2016, 0245_IBEROS_1_E, EAPA_151/2016, NORTE-08-5369-FSE-000044 and IF/00347/2015

REFERENCES:Diogo, G.S et al.,Marine drugs 2018, 16, (8), 269.

Keywords: Bone and bone disorders (osteoporosis etc),



Collagen-Induced Arthritis as a model to study bone injury in inflammatory conditions

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INTRODUCTION:In healthy conditions bone tissue is able to regenerate to its original architecture, through three coordinated stages: inflammation, repair and remodelling. While acute inflammation is required for complete repair, chronic inflammation has a major impact on bone and joint health. Rheumatoid arthritis (RA) is a systemic osteoarticular inflammatory disease, that is associated with increased bone fragility and risk of fractures. Herein, the aim was to study the systemic acute response to bone injury in chronic inflammatory conditions. The impact of chronic inflammation on endogenous mesenchymal stem/stromal cells (MSC) was also investigated.

METHODS:Collagen-Induced Arthritis (CIA) rat model was induced, and scored for arthritis progression. Critical size femoral bone defect was performed, and 3 days after animals were sacrificed. Cell proliferation in secondary lymphoid organs was evaluated, and systemic immune cells proportions in blood, draining lymph nodes and spleen, were analysed using multicolor flow cytometry. Plasma cytokine/chemokine levels were profiled using an array. Bone marrow MSC from CIA animais were probed for proliferation, metabolic activity, and differentiation.

RESULTS:Arthritis score and hind paw swelling were significantly increased, while secondary lymphoid organs, spleen and lymph nodes, were enlarged and their cells significantly more proliferative in CIA animals. Proportions of lymphoid cell populations were altered in spleen and lymph nodes of CIA animals, and myeloid cells were increased in blood. Upon bone injury in CIA animals, myeloid cell proportions, and their expression of co-stimulatory molecules (CD40 and CD86) were increased in spleen and lymph nodes. Screening plasma cytokine/chemokine levels showed increased inflammatory cytokines (e.g.TNF- α, IL-17, IL-12) in CIA, and IL-2 and IL-6 in CIA and CIA with bone injury, while Fractalkine and Leptin were decreased in both groups. CIA-derived MSC showed lower metabolic activity and proliferation, and significantly increased osteogenic and chondrogenic differentiation markers. Exposure of control-MSC to TNF-α partially mimicked the CIA-MSC phenotype in vitro(1).

DISCUSSION & CONCLUSIONS:The CIA animals had an inflammatory profile, but were still able to respond to bone injury, increasing their myeloid cell proportions/activation in secondary lymphoid organs, thus the combined model can be used to study the mechanisms of bone repair in inflammatory conditions. Finally, endogenous CIA-MSC were more prone to differentiation in basal conditions.

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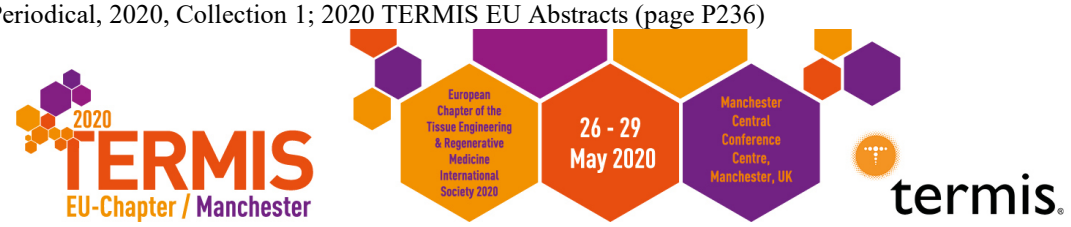
Keywords: Bone and bone disorders (osteoporosis etc), Cartilage / joint and arthritic conditions



Intrapericardial Injection of Hydrogels Derived From Decellularized Cardiac Extracellular Matrix Loaded With Mesenchymal Stromal Cells and Their Secretome: A Novel Proposal of Therapeutic Approach to Cytostatics-induced Dilated Cardiomyopathy

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INTRODUCTION:Intramyocardial injection (IM) of hydrogels containing stem cells, their secretome or both, may hold promise to treat dilated cardiomyopathy (DCM). This therapy, however, may lead to adverse outcomes, such as arrhythmias, related to the trauma of the IM injection and poor conductibility of the biomaterial. Additionally, DCM is a multichambered disease, which demands a treatment setup that reaches the entire heart. Thus, the optimal route of administration for cardiac cell therapy in DCM remains a challenge. We hypothesized that the intrapericardial injection of hydrogels derived from cardiac decellularized extracellular matrix (dECM) loaded with adipose tissue-derived stromal cells (ASC) and their secretome (conditioned medium, CMed) dampen or reverse the progression of DCM.

METHODS:DCM was induced in rats through ten weekly intraperitoneal injections of doxorubicin (cumulative dose: 18mg/kg). In week five, the animals were divided in intrapericardial treatments (2ml/kg): 1) saline, 2) dECM hydrogel and 3) dECM hydrogel loaded with ASC and their CMed. ASC concentration was 20 million per mL while 100x concentrated CMed in hydrogel were used. Non-treated, healthy rats, were used as controls. Interstitial myocardial fibrosis was determined by Sirius Red and hemodynamic parameters were determined by pressure-volume loops.

RESULTS:Interstitial myocardial fibrosis was reduced in ASC/CMed-treated animals compared to saline controls ($p=0.0139$). Ejection fraction and cardiac work efficiency were improved in the ASC/CMed-treated rats compared to saline ($p=0.0151$ and $p=0.0655$, respectively). Treatment with sole dECM hydrogel did not reduce fibrosis nor improve hemodynamic parameters.

DISCUSSION & CONCLUSIONS:The intrapericardial injection of dECM hydrogels loaded with ASC and their secretome warrant a novel therapeutic possibility by improving ventricular hemodynamics and reducing cardiac remodeling in doxorubicin-induced DCM.

Keywords: Cardiovascular, Hydrogels and injectable systems

Carbon nanotube based composite scaffolds and electrostimulation therapy for bone repair

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INTRODUCTION:Critical bone defects are considered one of the greatest clinical challenges in reconstructive bone surgery due to the limited regeneration capabilities of bone. Healthy bone tissue has the ability to generate endogenous electrical signals that stimulate the repair process after bone loss. The electrical stimulation therapy is reported to stimulate and accelerate the regeneration process, compensating the lack of endogenous electrical signals. Moreover, the application of “smart” electrically conductive scaffolds may favour adequate electrical stimulus to the injured bone. Polycaprolactone-based (PCL) three-dimensional biomaterials (scaffolds) associated with carbon nanotubes (CNTs) have been considered as a potential scaffold for bone tissue engineering. The combination of electroconductive materials and exogenous electrical stimulation at physiological level might act on cellular and molecular signalling pathways, leading to bone regeneration. This paper investigates the use of electrostimulation (ES) and PCL scaffolds produced with CNTs for bone regeneration.

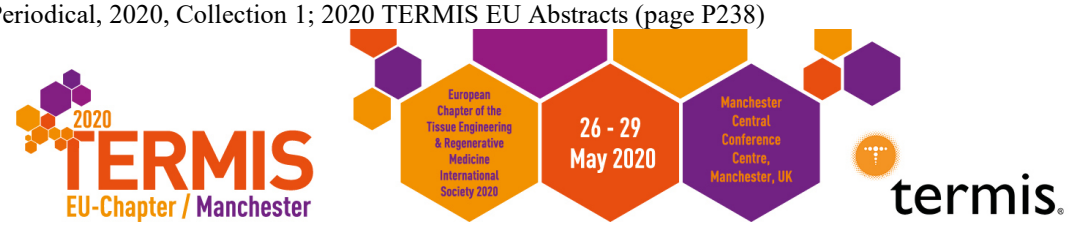
METHODS:The experimental protocol was approved by the animal ethical committee (002/2018). Critical bone defects (5mmx5mm) in wistar rats model were treated with ES (10µA/5min, twice a week) and ES associated to PCL-CNT scaffolds (0.75% and 3% in weight). Scaffolds (lay-dawn-pattern of 0/90º) were produced using a screw-assisted extrusion-based additive manufacturing system. Bone regeneration was evaluated on the 60th and 120th days by histomorphometric (blood vessels, mineralized tissue formation, bone remodelling phase (RANKL/OPG/TRAP) and protein expression (BMP-7 and collagen I) analyses. Data were processed using GraphPad_Prism software and analyzed by two-way ANOVA wit Bonferroni's test(α=5% significance level).

RESULTS:The higher number of blood vessels and increased mineralized tissue formation were observed when CNT scaffolds (0.75% and 3%) were used together with electrostimulation for both periods. The bone remodelling phase was more evident in all groups treated with scaffold+ES. BMP-7 expression in the CNT 3%+ES group was higher when compared to the ES and CNT 3% groups. The expression of COL-1 was higher for CNT 3%+ES group when compared to the SHAM and ES control groups.

DISCUSSION & CONCLUSIONS:The combined use of electrostimulation and CNTs scaffolds enhanced the bone repair process by increasing angiogenesis, tissue mineralization and the remodelling phase. BMP-7, responsible for inducing osteoblastic differentiation and leading to tissue formation and mineralization, was higher in the case of CNT3% and CTN3%+ES groups. Results suggest that the use of 3% CNT scaffolds produced by additive manufacturing combined with electrostimulation is a promising strategy to treat critical size bone defects.

ACKNOWLEDGEMENTS:Hermínio Ometto Foundation(Brazil), and The School of Mechanical, Aerospace and Civil Engineering, University of Manchester(UK).

Keywords: Additive manufacturing, Composite materials



Tissue engineered urinary conduit – preclinical study in a porcine model

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INTRODUCTION:The use of ileal segment is a standard method for urinary diversion after radical cystectomy. The main disadvantages of this method are a necessity of carrying out an additional surgical procedure and different properties of intestinal and urinary bladder tissues. To overcome these side effects, tissue engineering methods can be utilized to create the artificial conduit for urinary diversion. The aim of this study was to assess the tissue-engineered conduit for urinary diversion in a porcine model.

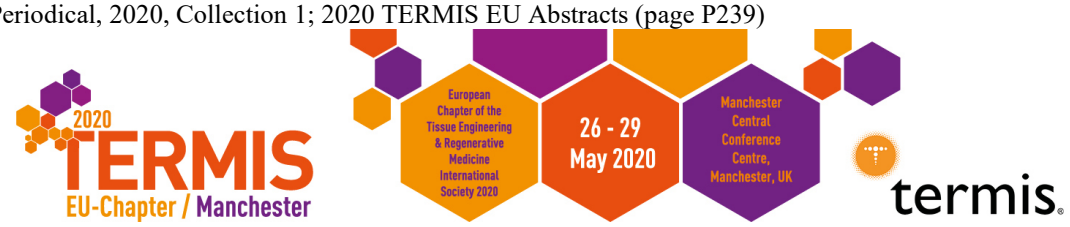
METHODS:Tissue engineered tubular scaffolds were used for construction of the artificial urinary conduits. Conduits were implanted as an incontinent urostomy using right ureters. Fifteen male pigs were used. In the 1st Group (Control, n=5) ureterocutaneostomy was created. In the 2nd Group (n=5) the right ureter- artificial conduit-skin anastomoses (the artificial conduit model) were performed. In the 3rd Group (n=5) 4 weeks before urostomy, the artificial conduit was implanted between abdomen muscles. The observation time was six months. Computed tomography was used to confirm the patency of created diversions. Morphological and histological analyses were used to evaluate the tissue-engineered urinary diversion.

RESULTS:All animals survived the experimental procedures and 6-months follow-up. The patency of ureterocutaneostomy (1st Group) was between 3 to 12 weeks compared to 18- 22 weeks for artificial conduit (2nd Group) and preimplantation procedure (3rd Group) 20-21. In the case of the 2nd Group the prolapse of tissue-engineered conduit was observed between 3 and 4 weeks after surgical procedure however in the 3rd Group the conduit loss process from 4 to 8 weeks was noticed. The remnants of the implant created a retroperitoneal post-inflammation tunnel which constitutes urostomy. Computed tomography and histological evaluation showed that the prolapse of a tissue engineered scaffold was related to the disruption of the scaffold integration process with adjacent tissues as a result of developing biomaterial infection.

DISCUSSION & CONCLUSIONS:The simultaneous urinary diversion using tissue-engineered scaffold connected directly with the skin is not appropriate method for clinical application, despite appearance of post inflammation tunnel. However, the scaffold implantation surgery 4 weeks before ureter reconstruction, extends the maintenance period and patency of the tunnel. Our results showed that there is emerging need for searching a new method solving the urinary diversion after cystectomy.

Acknowledgements:The present work was supported by the National Centre for Research and Development (NCBR) in Poland under Agreement no. STRATEGMED1/235368/8/NCBR/2014 (Smart AUCI Project) within the Strategic Programme STRATEGMED “Prevention practices and treatment of civilization diseases.”

Keywords: In vivo and animal models, Biomaterials



Advanced ““in vitro““ and ““in vivo““ approach as effective drug screening platforms for cells and tissue-engineered medical products: Accelera’s experience

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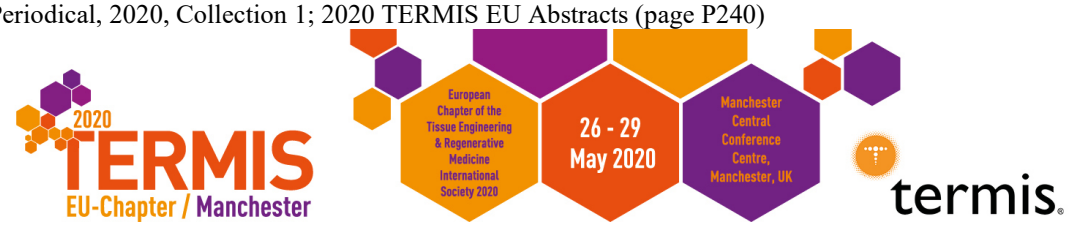
INTRODUCTION:Despite the success of several clinical applications of cells and tissue-engineered medical products, numerous new products fail to account for the criteria that determine the translational success and lifespan of a product. Moreover, the diverse characteristics of advanced therapy medicinal products (cells and non-cells components for example) make the assessment of their tolerability and quality complex. Since safety and biocompatibility tests for all the product are mandatory, an adequate and fine-tuning modular experimental setup is pivotal to avoid erroneous decisions during drug development.

METHODS:In this poster, we will provide an overview of approaches that Accelera has adopted in the assessment of toxicity and ADME (Absorption, Distribution, Metabolism, Elimination) properties of different cell, gene, tissue engineered and regenerative medicine products. Evaluation of the stability of cellular/biomaterial phenotypes, methods for test item delivery and treatment localization are usually carried out combining in vitro testing (i.e cell culture) and in vivo assessment in animal models under good laboratory practice (GLP). Following the policy that promotes replacement, refinement, and reduction of animal experiments, the 3 R principle, promising new in vitro tools (i.e organ-on-chip) are suddenly explored to confirm data obtained with animal models, such as the absence of toxic events (e.g., by assessing cardiovascular, liver, or skin toxicity).

RESULTS:Each related problem in the components of the product usually is solved using different tools e.g.: - Autoradioluminography techniques and HPLC-MS/MS approach to determine tissue absorption, distribution metabolism and excretion of cellular and non-cellular materials; or to measure the interaction/binding of a product to specific device components to improve the development of new delivery systems.
- Molecular biology tools (RNA extraction, qPCR), microscopy (i.e immunofluorescence, immunohistochemistry), ELISA and cytofluorimetric analysis to track and monitor therapeutic cells in vivo; - Radiolabeling techniques applied to synthetic biomaterials, to improve research for regenerative medicine and prosthetic devices.

DISCUSSION & CONCLUSIONS:Finally, the advantages and disadvantages of our approach will be debated.

Keywords: Advanced therapy medicinal products, In vivo and animal models



Regenerative potential of Adipose-Derived Stromal Cells seeded on tissue engineered tubular scaffolds for urinary diversion – preliminary results in a porcine model

Arkadiusz JUNDZILL, Daria BALCERCZYK, Tomasz KLOSKOWSKI, Monika BUHL, Kamil SZELISKI, Marta RASMUS, Tomasz DREWA, Marta POKRYWCZYNSKA
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INTRODUCTION:Standard method of urinary diversion following radical cystectomy is use of ileal conduit which is associated with numerous metabolic complications. Tissue engineering methods make it possible to construct implants that can improve the patient's quality of life. The aim of this study was to evaluate how adipose-derived stromal cells (ADSCs) influence on tissue engineered conduit integration with host tissues in a porcine model.

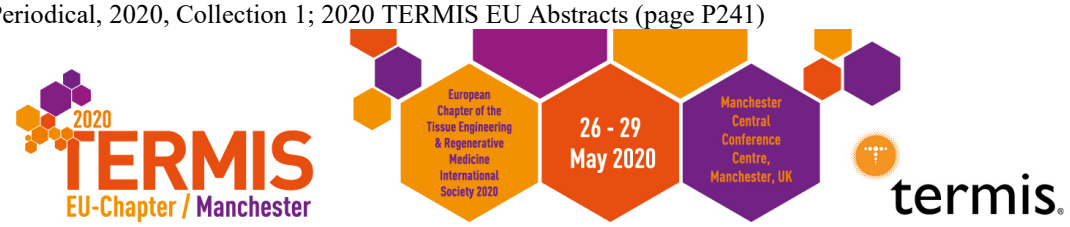
METHODS:Six male pigs were used in the experiment. In the 1st Group (n=3) isolated and then propagated autologous porcine ADSCs were seeded on the artificial conduit, which was then cultured for 7 days under controlled bioreactor conditions. Prepared tissue-engineered conduits were implanted between the abdomen muscles. The immunophenotype of isolated cells was analyzed by flow cytometry (CD29+, CD90+, CD44+, CD31-, CD11b-, CD45-). To track cells in vivo, PKH26 cell labeling method was used. In the 2nd Group (n=3) intramuscular implantation of unseeded conduits was performed. The observation time was three months. Morphological and histological analyses were used to evaluate the integration of implanted conduits.

RESULTS:All animals survived the experimental procedures and 3-months follow-up. Morphological observations and histological staining of implanted conduits showed reduction of inflammation and fibrosis which resulted in increased scaffold integration in the 1st Group compared to 2nd Group. PKH26 stained cells were observed mainly in the center of implanted conduits. Single stained cells were observed in the distal and proximal parts of the scaffold.

DISCUSSION & CONCLUSIONS:Presented results showed that the use of artificial conduit in combination with Adipose-Derived Stromal Cells significantly improves the integration of the implant with host tissue. These results can be beneficial for developing a new method of urinary diversion after radical cystectomy.

ACKNOWLEDGEMENTS:The present work was supported by the National Centre for Research and Development (NCBR) in Poland under Agreement no. STRATEGMED1/235368/8/NCBR/2014 (Smart AUCI Project) within the Strategic Programme STRATEGMED “Prevention practices and treatment of civilization diseases.”

Keywords: Multipotent (mesenchymal) stem cells, Bioreactors



Phenotypic correlates of cartilage tissue yield: a comparison of iPS cells with MSCs

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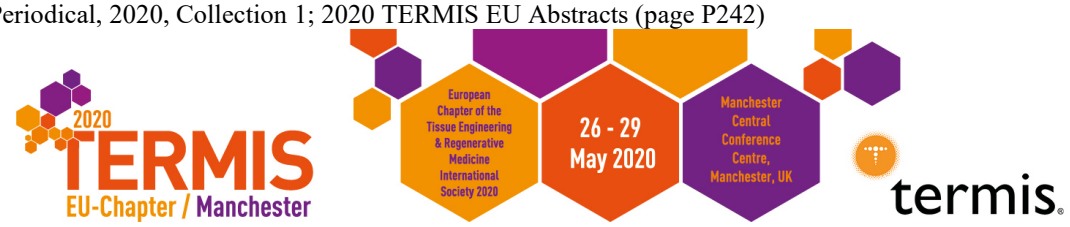
INTRODUCTION:Chondrocyte- or mesenchymal stromal cell (MSC)-based therapies remain inefficient in restoring biochemical and biomechanical properties of hyaline cartilage upon injury. Induced pluripotent stem cells (iPSCs), characterized by high proliferation and differentiation potential, are a promising alternative cell source for generating human articular chondrocytes that could be used for cartilage regeneration, drug screening and disease modelling. However, significant cell loss during the initial phase of chondrogenesis remains a bottleneck in generating cartilage from iPSC-derived mesenchymal progenitors (iMPC). In contrast, MSCs survive better during chondrogenesis, albeit with propensity to undergo endochondral differentiation, resulting in inferior cartilage tissue quality. Aim of this study was to identify mechanisms and correlates of the low cartilage tissue yield from iPSC chondrogenesis.

METHODS:Expanded MSCs and iMPCs were subjected to global gene expression analysis by Illumina cDNA microarray. The results were analyzed using bioinformatics and differential gene expression patterns were confirmed via qPCR. Safranin O staining and immunohistochemistry were used to assess proteoglycan deposition and pellet size on day 42 of chondrogenesis in vitro. Cell survival rate was determined via PicoGreen DNA quantitation assay. Differential cell adhesion properties were tested in a condensation assay under chondrogenic conditions and the activity of extracellular-regulated kinase 1/2 (ERK1/2) signaling of cells cultured either in suspension or in agarose was analyzed by Western blotting.

RESULTS:Comparison of the transcriptomes of the two cell types showed that iMPCs had higher expression of early mesodermal markers suggesting a more juvenile phenotype than MSCs. Furthermore, iMPCs had less myofibroblast-like characteristics, indicated by lower α-smooth-muscle-actin expression as well as significantly lower extracellular matrix (ECM)- and integrin-pathway-related gene expression, including decorin, collagen VI, lumican and laminin. A high cell density condensation assay demonstrated that iMPC-derived aggregates were less cohesive and large aggregate formation was impaired compared to MSCs. Importantly, this correlated with lower ERK1/2 pathway activity in iMPCs vs MSCs. High active ERK1/2 signaling in MSCs was shown to be adhesion-dependent.

DISCUSSION & CONCLUSIONS:Inferior cell cohesiveness, scarce ECM production and consequent loss of a non-aggregating cell fraction led to a significant cell loss during early iMPC chondrogenesis in vitro. Moreover, iMPC survival was also compromised by low pro-survival ERK1/2 activity upon pellet formation during chondrogenesis. Taken together, we conclude that low expression of ECM molecules, insufficient pro-survival ERK1/2 signaling and inferior cell cohesiveness were correlates of cartilage tissue yield from iPSCs.

Keywords: Cartilage / joint and arthritic conditions, Multipotent (mesenchymal) stem cells



Chondrogenic differentiation of human induced pluripotent stem cells (iPSc) for disease modelling and regenerative medicine purposes

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INTRODUCTION:Induced pluripotent stem cells (iPSc) are considered to be promising cell sources for biomedical applications. Excellent chondrogenic differentiation capacity is the hallmark of a potential cell source for both treating cartilage lesions and for in vitro studying molecular mechanisms of cartilage diseases, such as osteoarthritis. Nevertheless, efficient protocols to chondrogenically differentiate iPSc are still required. For this reason, the purpose of this study has been to compare three different strategies to differentiate iPSc into chondrocyte-like cells.

METHODS:The iPSC-line N1-FiPS4F#7, which previously established by our group from a donor without rheumatic diseases, was used (Castro-Viñuelas et al., 2019). Three chondrogenic protocols were tested: (1) directed differentiation (2) differentiation by means of embryoid bodies (EBs) formation and sequential addition of growth factors, and (3) differentiation throw a mesenchymal stromal cell (MSC)-like state followed by micromass formation and culture in chondrogenic medium. In order to evaluate whether MSC-like cells were obtained in the intermediate state of the protocol number 3, cells were morphological, phenotypical and functionally characterised. Finally, chondrogenic differentiation was evaluated by histological techniques for visualization of typical cartilage extracellular matrix proteins.

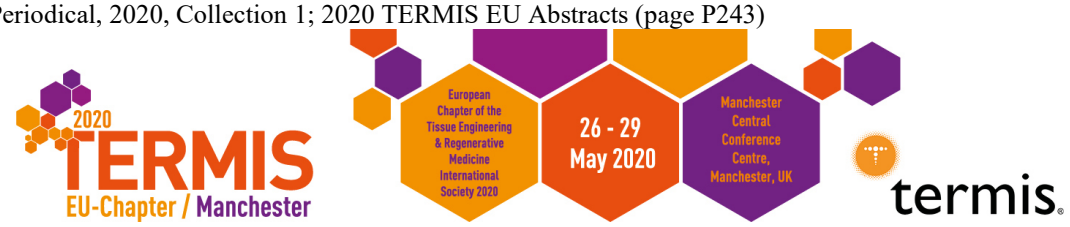
RESULTS:MSC-like cells were obtained during the protocol number 3, as demonstrated by cell morphology, expression of specific surface markers (CD29, CD73, CD90, CD105) and capacity to differentiate into adipogenic, osteogenic and chondrogenic lineages. After performing each protocol, Masson's trichrome and Safranin O staining revealed that the formation of EBs followed by the sequential addition of growth factors (protocol 2) allowed the highest levels of extracellular matrix components production within the micromasses. On the other hand, micromasses obtained after protocol 3 showed presence of collagen fibres but less levels of proteoglycans inside the matrix. Finally, regarding protocol number 1, iPSc formed spontaneous micromasses by day 21 of the differentiation, but neither collagen nor proteoglycans were detected histologically in those micromasses after finishing the directed differentiation protocol.

DISCUSSION & CONCLUSIONS:Among the three protocols tested in this research, just the differentiation protocol based on the formation of EBs and the sequential addition of growth factors enables the differentiation of the iPSc into chondrogenic-like cells. These results should contribute to elucidate the best approach to obtain chondrocytes from iPSc, a crucial step in translating the use of these pluripotent cells to clinical applications.

Acknowledgements:MPR, SRF and CSR are granted by Xunta de Galicia and European Union (European Social Fund).

References:Castro-Viñuelas, R et al. (2019). Stem Cell Research. In press.

Keywords: Differentiation, Disease models



Ex vivo infected wound model based on viable human skin for testing novel antimicrobial treatments

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INTRODUCTION:Open wounds lead to a loss of barrier function of the skin and an increased risk of microbial colonization and infection. The current threat of antibiotic resistance coupled with slow development of novel antimicrobial treatments calls for new methods for the evaluation of potential therapeutics. The aim of the present study was to develop a standardized ex vivo infected wound model based on viable human skin. The model was subsequently used to evaluate antimicrobial treatment in regard to reduction of infection.

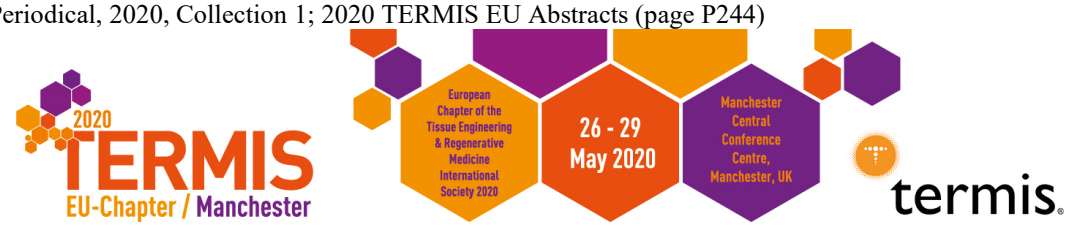
METHODS:Skin was obtained from healthy patients undergoing routine plastic surgery (Swedish Ethical Review Authority no. 2018/97-31). Following removal of subcutaneous fat and large blood vessels, circular full-thickness skin disks (8 mm diameter) with a central partial-thickness wound (4 mm diameter) were created using biopsy punches. The wounds were inoculated with 10^7 Staphylococcus aureus (ATCC 29213), and maintained under standard cell/tissue conditions up to twelve days. Tissue infection was monitored using quantitative cultures on selective media as well as immunohistochemical staining using primary antibodies directed towards S. aureus and pancytokeratin. Reepithelialization was evaluated using routine histology. Moreover, topical gentamicin administration (1 mg/ml) was evaluated in regard to eradication of infection using the infected wound model.

RESULTS:Quantitative cultures revealed a wound infection three days after inoculation of approximately 10^7 colony forming units (CFU)/g of tissue, increasing to 10^9 CFU/g twelve days after inoculation. Immunohistochemical staining confirmed the bacteria to be S. aureus, and revealed the spread of infection down into the dermis. No sign of reepithelization was observed in the infected wounds twelve days post inoculation, whereas non-infected control wounds were completely covered with neoepidermis, as confirmed using antibodies against pancytokeratin. Antimicrobial treatment using topical gentamicin completely eradicated the wound infection.

DISCUSSION & CONCLUSIONS:The current study describes the establishment of an infected ex vivo wound model based on viable human skin. The model is reproducible and provides rapid screening of novel antimicrobial treatments, assessing reduction of infection as well as reepithelialization over time. Further development of the infected wound model will include other clinically relevant pathogens, as well as biofilm formation.

Acknowledgements:The project received financial support from the Kamprad Family Foundation, ALF-Region Östergötland and the Swedish Foundation for Strategic Research. The authors thank A Starkenberg and K Briheim for excellent technical assistance.

Keywords: Wound healing, Skin



Use of Cryopreserved Human Aorta as a Tracheal Connector for the Development of an Artificial Larynx

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INTRODUCTION:Total laryngectomy is a life saving procedure for end stage laryngeal cancers, but it significantly decreases the patient's quality of life with significant complications. An artificial larynx system that can take over the functions of the larynx after resection could be a potential solution. Previously we have developed an artificial larynx that can partially restore laryngeal functions up to 18 months (1) but the integration with the surrounding tissues was slow. Herein, we aim to improve the integration of the connection between the artificial larynx and the remaining trachea by using cryopreserved human aortas as a tissue connector.

METHODS:6 sheep were implanted with cryopreserved aortas in a manner that establishes a connection between the base of the tongue and the tracheal rings; to demonstrate the feasibility of the approach with a follow-up period of 6 months. In order to monitor the epithelialisation, lumens of aortas were seeded with human respiratory epithelial cells and monitored up until 14 days in vitro (SEM and Confocal microscopy). In order to induce faster cellular in-growth, aortas were lyophilised and seeded with human stem cells and monitored for cellular proliferation and chondrogenic differentiation (GAG production and chondrogenic gene expression by RT-PCR) in vitro.

RESULTS:5 out of 6 sheep had the grafts in place after 6 months without any necrosis but with limited epithelialisation. In vitro, the luminal surface of the aortas was conducive to the adhesion of respiratory epithelial cells and formation of a continous epithelial layer.(as demonstrated by an increased ZO-1 expression and coverage of the surface). Lyophilisation of the aortas resulted in a porous environment with better cellular infiltration, increased GAG secretion and chondrogenic differentiation related gene expression in the presence of TGF-beta.

DISCUSSION & CONCLUSIONS:Our preliminary studies in vitro and in vivo demonstrated the feasibility of utilisation of cryopreserved aortas as a tracheal tissue connector. Our future work will focus on the optimisation of the conditions for functional epithelialisation and chondrogenesis.

ACKNOWLEDGEMENTS:This research received funding from the Institut Carnot MICA (Matrix Reloaded project) and from the European Union’s H2020 programme, through PANBioRA project.

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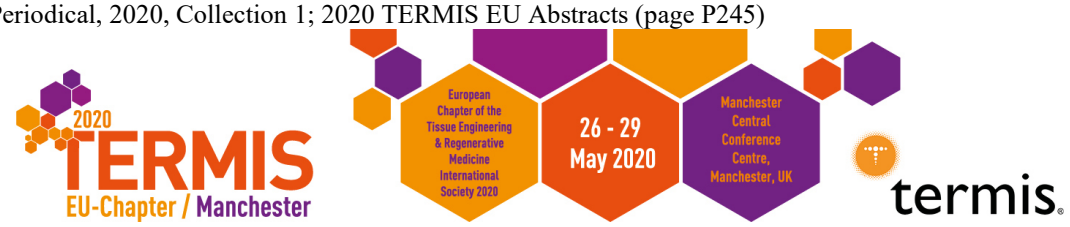
Keywords: Interfaces - biological, In vivo and animal models



Development of musculoskeletal interfaces

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INTRODUCTION:Tissue interfaces are transition zones located between different tissues, composed of elements derived from the tissues they connect. In the musculoskeletal system, interfaces are crucial for load transfer from one tissue to another (1). Recently, the interest in interface development has increased as their inability to regenerate leads to compartmentalisation, zoning off, pain and organ malfunction, together with graft instability and limited implant-host integration (2).
The aim of this research is to develop a 3D in vitro model that combines bone, tendon and muscle. The development of the interfaces between these tissues was evaluated, focusing on the behaviour of the cells. Experiments were conducted in a growth chamber that allowed for the separation of medium but maintained cell communication.

METHODS:Osteoblast-like cells (MG-63), human dermal fibroblasts (HDF) and skeletal muscle cells (Sket.4U) were investigated individually, to determine proliferation rate, metabolic activity, morphology and cell type specific marker expression. Different culture conditions were tested in 2D and 3D culture for 1, 3, 7 and 14 days. Metabolic activity was detected using Alamar blue and MTT, DNA content was measured with PicoGreen and the morphology was determined using histology. Experiments were performed with different cell densities to determine the most suitable to reach homeostasis in the 3D model. Moreover, the results were compared using gels made of collagen or alginate. The 3D model was fabricated using type I collagen by indirect 3D printing and was composed of three phases with different stiffness, composition and shape.

RESULTS:Sket.4U demonstrated lower metabolic activity compared to the other cell types. Cells seeded as monolayers showed an increased level of metabolic activity over 14 days. In 3D, this rate was comparable for low cell densities, but for the highest cell densities the metabolic activity decreased after 14 days. A similar trend was observed for the DNA content.

DISCUSSION & CONCLUSIONS:The cells' morphology in monolayers showed that MG-63 organised in nodules after day 7 and the HDF were oriented. Sket.4U did not show the characteristic organisation in fibres, suggesting a need for a higher seed density.
Consequently, a cell density of 1,000,000 cells/ml was chosen for MG-63 and HDF, while 2,000,000 cells/ml were chosen for Sket.4U.

References:1. Yang PJ, Temenoff JS. Tissue Eng Part B Rev. 2009;15(2):127-141.
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Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Interfaces – biological

Effects of Biomimetic Xeno-Free Protein Repulsive Surface On Human Embryonal Stem Cells

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INTRODUCTION:The interaction between the human embryonal stem cells (HESc) and their environment is vital for their migration and differentiation during the embryonic development and morphogenesis. The study of these specific interactions is important. Stem cell niche is represented by proteins of the extracellular matrix, cell junctions and soluble factors. Cell culture surfaces modified by synthetically prepared peptide motives (cell surface receptors ligands) can provide unique platform for studies of single molecule-ligand influence on cell behavior.

METHODS:To prepare surfaces, we have developed and utilized a system combining the covalent bond of peptides to linkers applying click chemistry and a protein-repulsive surface made poly(2-hydroxyethyl methacrylate) based polymer (pHEMA).

RESULTS:The pHEMA surface retains its protein repulsive properties after biomimetic modification1 and therefore lowers the probability of false positive results by preventing the non-specific adhesion of cells on adsorbed proteins from the culture medium.

DISCUSSION & CONCLUSIONS:We analyzed the response of HESc to synthetic peptides and to characterize molecular pathways. This knowledge can be utilized for cell culture expansion and creating relevant models for disease modelling and developmental studies.

Acknowledgements:The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic within the National Sustainability Program I (NPU I), Project POLYMAT LO1507.

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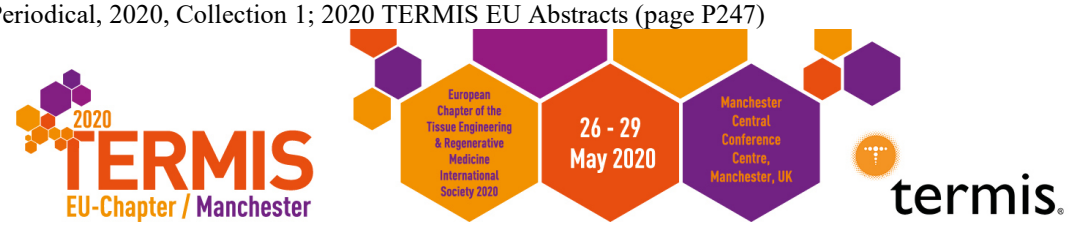
Keywords: Interfaces - engineered, Stem cells – general



Trilineage Potency of Human Nucleus Pulposus Cells before and after Cryo-Preservation

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INTRODUCTION:Low back pain (LBP) is a major cause of disability in many countries, affecting more than half a billion people worldwide. A promising and future-oriented approach to treat LBP is cell therapy using stem or progenitor cells. Over the last decade, cells positive for Tie2 and mesenchymal stromal cell markers have been found within the nucleus pulposus (NP) of human intervertebral discs (IVD). However, little is known about the effect of expansion and cryo-preservation on here called “heterogenic” human NP cells (hNPCs) and their stemness in a context of cell therapy for regeneration of the IVD. Therefore, the aim of our study was to expand hNPCs whilst investigating their differentiation potential before and after cryo-preservation and to find and optimal approach to cryo-preserve them.

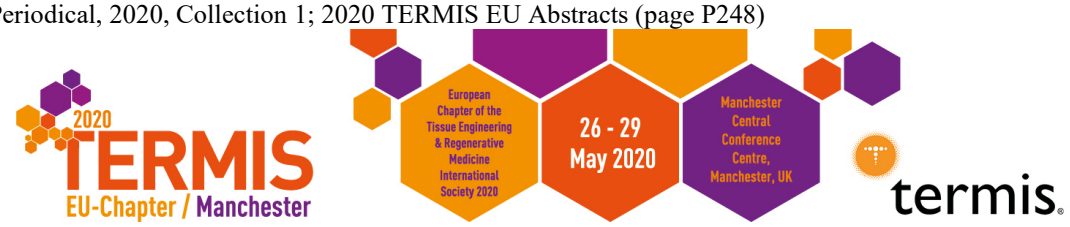
METHODS:HNPCs from three human trauma patients (32, 55 and 69 years old) undergoing spinal surgery were isolated with a mild two-step digestion protocol. After subsequent expansion until complete confluency, hNPCs were separated and then differentiated into osteogenic, adipogenic or chondrogenic lineages for 21 days or were cryo-preserved for one week at -150°C with five cryo-preservation media (90% fetal bovine serum and 10% dimethyl sulfoxide (DMSO); 90% low glucose medium + 10% DMSO and three commercially available media) to compare their effect on the cell’s viability and differentiation potential. Cell viability was determined with trypan blue and by cytometry employing propidium iodide. The differentiation potential was assessed using histological analysis and qPCR.

RESULTS:HNPCs cultured in osteogenic medium showed a significant ($p<0.01$) higher expression of calcium deposits (up to 11-fold) vs. controls, indicating osteogenic differentiation. Furthermore, evidence for adipogenic and chondrogenic differentiation was observed using histological analysis and determining genes typical for chondrogenic and adipogenic lineages like collagen type 2 (up to 350-fold) or adiponectin (up to 3’700-fold). In addition, most hNPCs maintained their differentiation potential, even after cryo-preservation and independent of the cryo-preservation medium used. The hNPCs’ cell viability after storing for one week at -150°C was very similar for all conditions (~85% cell viability).

DISCUSSION & CONCLUSIONS:The study showed heterogenic hNPCs have trilineage potential and as such possess stem cell characteristics. Therefore, they can potentially be used for future clinical trials concerning cell therapy for IVD regeneration. Furthermore, commercially available cryo-preservation media seem to perform just as well as homemade media in terms of cell viability and maintaining hNPCs differentiation potential.

Acknowledgements:Financial support was received from iPSpine H2020 project #825925.

Keywords: Intervertebral disc / spine and their disorders, Cell therapy



Spheroid-like Cultures for Cell Expansion of Angiopoietin Receptor-1 (aka. Tie2) positive Cells from the human Intervertebral Disc

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INTRODUCTION:Low back pain is the leading cause of disability worldwide (1). Nevertheless, the mechanism of the intervertebral disc (IVD) degeneration is still not clear. In this context, the nucleus pulposus (NP) and more precisely NP progenitor cells (NPPCs) present in the IVD, positive for angiopoietin-1 receptor (aka. Tie2) display multipotent and stem capacity (2,3). In this study, the first aim was to determine whether spheroid formation in suspension-culture will increase the amount/percentage of NPPCs during the expansion compared to traditional monolayer culture. The second aim of this study was to investigate if the percentage of NPPCs will be enriched even further by the resuspension of the spheroid-like cultured cells (=1st generation) and reformation of those spheroids one more time (= 2nd generation).

METHODS:Human NP tissues from trauma patients (N=3) were obtained with written ethical consent and isolated by a two-step digestion protocol (3). The NP cells were resuspended and frozen at -150°C after reaching confluence of passage 0. At passage 1, NP cells were seeded in standard or ultra-low attachment tissue culture flasks with 2.5 ng/ml FGF-2 in low glucose - DMEM (supplemented with 10 % FBS). Flow cytometry was used to analyze and quantify the percentage of NPPCs using Tie2 antibody. We defined the spheroids formed after passage 1 NPCs as 1st generation spheroid. We obtained the 2nd generation spheroids by resuspending the 1st-generation-spheroid and reassembly. The NPCs from 1st and 2nd spheroid were quantified by CFU-assay.

RESULTS:As a result, the percentage of NPPCs in monolayer culture condition was reaching 7 ± 2 % (Mean±SEM), however, in the 1st and 2nd generation spheroids culture condition, we were observing 20 ± 10 % and 28 ± 6% of Tie2+ cells, respectively. Concerning the CFU-assay, the NPCs from the 2nd generation spheroid formed 30 CFU-S per 1,000 cells, which were twice more CFU-S compared to the 1st generation spheroid. DISCUSSION & CONCLUSIONS:From these data we conclude than the spheroid-like formation of NPCs would be a more efficient method for expansion and enrichment of NPPCs than monolayer expansion in a context of future cell therapy.

Acknowledgements:Financial support was received from iPSpine H2020 project under grant agreement #825925 and China Scholarship Council to X.Z.

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Keywords: Intervertebral disc / spine and their disorders, Stem cells – general

Extracellular Matrix-Mimetic Collagen/Hyaluronan Hydrogels to Promote Stem Cell Differentiation for the Treatment of the Nucleus Pulposus Degeneration

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INTRODUCTION:Half of chronic back pains is associated with intervertebral disc (IVD) degeneration. Novel treatments relying on stem cells injection have emerged with the aim of regenerating the Nucleus Pulposus (NP). Unfortunately, the results were disappointing due to the lack of cell differentiation. As the cell behavior depends on the biochemical, mechanical and topological environment, we hypothesized that a biomimetic hydrogel would promote IVD regeneration by providing cues to cells to differentiate into nucleopulpocytes. For this purpose, a panel of hydrogels with different mechanical properties, hydration degree and biochemical compositions were studied to analyze their impact on stem cell differentiation.

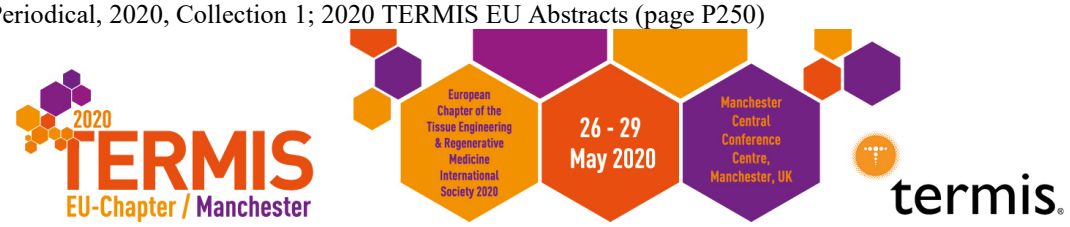
METHODS:Composite hydrogels were synthesized by co-gelling of Collagen (Col) and a tyramine Hyaluronan derivative (HA-Tyr). Collagen gelling was triggered by pH increase and HA-Tyr chemical gelling was based on an enzymatic crosslinking by Horseradish Peroxidase (HRP) and H_2O_2. Increasing HA-Tyr contents up to 2 % and different HRP and H_2O_2 were used to modulate the kinetic and the degree of crosslinking. Col/HA-Tyr hydrogels were characterized using scanning and transmission electron microscopy, rheological measurement, DSC, in vitro degradation and swelling experiments. Bone Marrow Mesenchymal Stem Cells (BM-MSCs) or Stem cells from Human Exfoliated Deciduous teeth (SHEDs) were encapsulated within Col/HA-Tyr with different HA contents and cultivated over 28 days. Cell viability was assessed and cell differentiation analyzed by real time PCR and indirect immunohistochemistry. Several markers of nucleopulpocytes differentiation, such as type II Collagen, aggrecan and KRT 18/19 were monitored.

RESULTS:The physico-chemical study showed that a high HA-Tyr content and a fast gelling were required to positively impact the physical properties of hydrogels and mimic the ECM environment of NP. The fabrication process allowed the generation of highly hydrated hydrogels (90%), resistant against enzymatic degradation in which collagen fibrillogenesis was preserved. In addition, hydrogels exhibited mechanical properties similar to those of NP. A high HA-Tyr content positively impacted cell viability of BM-MSCs but not that of SHEDs. In addition, the presence of collagen was necessary for an adequate cell adhesion. Aggrecan was detected in BM-MSCs and SHEDs after 7 and 14 days, respectively. This evidenced the cell differentiation into nucleopulpocytes was faster with BM-MSCs.

DISCUSSION & CONCLUSIONS:This study shows that synthesizing a Col/HA hydrogel mimicking the composition and the ultrastructure of the natural NP allows the differentiation of different stem cells into nucleopulpocytes.

ACKNOWLEDGEMENTS:Financial support was received from the french “Ministère de l’enseignement supérieur et de la recherche”.

Keywords: Composite materials, Differentiation



The Influence of Dynamic Physiological Loading on a Papain-Induced Ex Vivo Model of Intervertebral Disc (IVD) Degeneration

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INTRODUCTION:Proteolytic enzyme digestion of the intervertebral disc (IVD) offers a method of creating a cavity to simulate IVD degeneration for the study of regeneration therapies. In this work, we developed a bovine organ culture model of IVD degeneration using the enzyme papain and assessed the influence of dynamic loading on matrix turnover and disc dimensions.

METHODS:Bovine caudal IVDs (n=9) were harvested from three donors (less than 24 months old). Whole IVDs were cultured according to previously established protocols [1]. Physiological loading was applied to the IVDs consisting of 2h of load, 0.02 – 0.2 MPa, and 0.2 Hz. After 7 consecutive days of loading, 100 µL of either PBS or 65 U/mL papain (Sigma Aldrich) was injected into the center of the explants. Subsequently, the papain (n=3) and PBS-injected (n=3) specimens continued to be loaded daily or were cultured under free swelling conditions (n=3). Disc height and diameter were measured immediately after dissection and before and after each loading cycle. After a total of 14 days of culture, the tissues were sampled for sulfated glycosaminoglycan (GAG) and gene expression with real time PCR to measure expression levels of COL1, COL2, ACAN, TAGLN, ELN, and ADAMTS5. Repeated disc height measures were analyzed with ANOVA, where statistical differences ($p<0.05$) were detected. Tukey's posthoc test was applied.

RESULTS:Both papain loaded and papain free-swelling specimens exhibited similar cavity formation in the nucleus pulposus (NP), lamellar disorganization in the annulus fibrosus (AF), and similar decreases in GAG content. However, daily physiological loading induced significantly higher rate of height and volume loss during the 7-day digestion period ($p<0.002$). Gene expression changes relative to day 0 showed an overall down-regulation of COL1, COL2, TAGLN, and ELN, while ADMTS5 was upregulated. ACAN was upregulated in the outer AF and downregulated in the inner AF.

DISCUSSION & CONCLUSIONS:This degeneration model will allow for injection of regenerative therapeutic approaches. Papain induced degenerative changes the bovine tissues, both under free swelling and loaded conditions. However, repetitive dynamic loading during enzymatic digestion induced more pronounced biomechanical and dimensional changes in the IVDs compared to free swelling conditions. In future work, transplanted cell behavior in the papain-induced degeneration model will be evaluated.

ACKNOWLEDGEMENTS:The work described is part of the iPSpine project, which has received funding from the European Union's Horizon 2020 Research and Innovation Programme (Grant No 825925).

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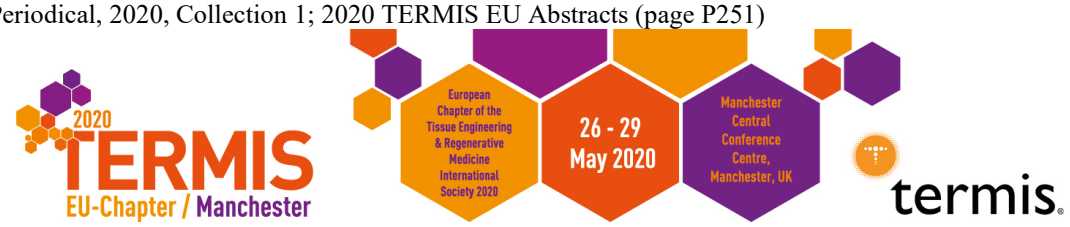
Keywords: Intervertebral disc / spine and their disorders, Disease models



Development of a bio-artificial kidney as a cell-based renal model

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INTRODUCTION:Understanding the active secretion of drugs by the kidney can be key for some drugs. Currently, cell lines expressing drug transporter proteins are applied for this purpose, however, in vitro- in vivo discrepancies are known to exist and these could be improved by mimicking the in vivo conditions of the renal proximal tubule cells where drug transporters are expressed (1).In order to mimic physiological conditions of renal proximal tubule, we have developed a custom-made module containing a single polypropylene hollow fibre that serves as a porous scaffold for cell attachment to generate a bioartificial kidney (BAK). In addition, a constant flow of media on both the lumenal and extracapillary space (ECS) exposes cells to shear stress while introducing fresh media and removing waste (2,3).

METHODS:The biocompatibility of the module was studied by seeding MDCK-Mdr1 or HEK-OCT2 cell lines overexpressing rat Mdr1a and OCT2 respectively in the HF lumen. Cell attachment and tight junction formation were evaluated by fluorescence microscopy and permeability assays. In addition, the transport activity of these cells was investigated using the fluorescent substrates Rho123, and ASP+ which are transported by P-gp, and OCT2 respectively and compared to cell lines cultured in conventional 2D models. Finally, qPCR of transporters along with cellular and renal markers has been used to compare gene expression changes between 2D and 3D cultured cells.

RESULTS:MDCK-MDR1 cells were shown to form a monolayer when exposed to shear stress in comparison to static conditions. HEK-OCT2 cells also cover the HF inner surface but as expected did not form tight junctions. In addition, qPCR results have shown significant changes in gene expression compared to cells grown in 2D. Basolateral markers such as Na/K ATPase, transporters P-gp and OCT2 and tight junction marker ZO-1 have shown a twofold gene downregulation compared to cells grown in static conditions, while the proliferation marker Ki-67 and the microvilli marker CD133 have shown a twofold increase. Finally, fluorescent substrate transport assays have confirmed a reduced transport activity compared to 2D assays.

DISCUSSION & CONCLUSIONS:These results indicate that the custom-made BAK device reduces transport activity in cell lines and influences gene expression compared to conventional cell cultures due to the 3D environment and shear stress. This will be examined further with the use of primary human renal proximal tubule epithelial cells (RPTEC) and their function in the BAK device.

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Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Kidney

Manufacturing a PCL electrospun device for knee ligament repair using traditional textile methods

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INTRODUCTION:Anterior Cruciate Ligament (ACL) tear is a devastating injury, common amongst young athletes. Current repair involves a surgical reconstruction of the ACL using hamstring or patellar tendon, in combination with sutures. This approach still yields unsatisfactory clinical outcomes, failing to restore adequate knee function in some patients. Furthermore, several synthetic ligaments tested have all failed due to insufficient mechanical properties and poor biocompatibility. [1] Consequently, there is a need to develop better alternatives, and we have manufactured a prototype of bioresorbable textile-based ACL scaffold, and tested its structure, strength, and safety against the scaffold made FiberWire®.

METHODS:Continuous electrospinning on metal wire was used to produce the building material for further manufacturing. [2] Electrospun polycaprolactone (PCL) filaments were stretched, with their final length of 7-8 times the starting length. Stretched filaments were used in continuous twisting to produce plied and cabled yarns. Subsequently, cabled yarns were used to produce a novel woven electrospun patch. The mechanical properties of the woven patch and individual filaments were measured by tensile tests. Scaffold morphology was assessed via SEM.

RESULTS:The SEM images showed a biomimetic morphology of the aligned microfibres, with the size of around 2 µm, similar to the ACL microstructure. Electrospun filaments showed enough strength to be used in twisting plied yarns (twisted in S direction) and cabled yarns (Z direction). Cabled yarns were weaved into a 10 mm wide plain pattern fabric, with a tensile strength of 272 ± 13 N (n = 10), comparable to FiberWire® with a tensile strength of 326 ± 14 N (n= 4).

DISCUSSION & CONCLUSIONS:For the first time, we have shown that continuous electrospun filaments made of PCL can be produced and be processed through several textile manufacturing steps, including drawing, twisting and weaving. This is a significant development in the field of functional bioresorbable medical textiles, in particular for applications in soft tissue repair such as knee ligament repair.

ACKNOWLEDGEMENTS:This research was funded by the Norman Collisson Foundation and the NIHR Oxford Biomedical Research Centre.

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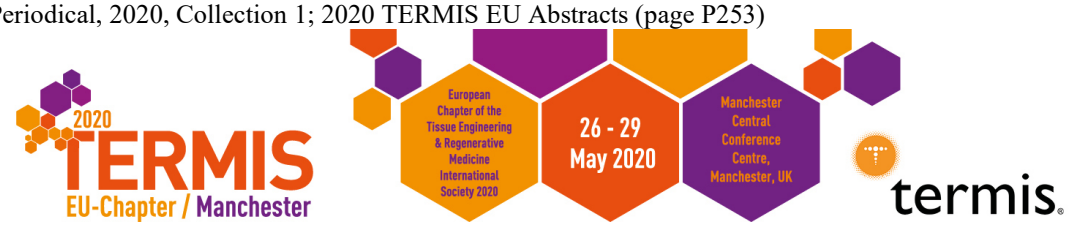
Keywords: Knee,



A 3D model for the survival niche of human long-lived bone marrow plasma cells

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INTRODUCTION:Human long-lived plasma cells (LLPCs) are terminally differentiated effector cells of the B-lymphocyte lineage that reside in specialized niches in the human bone marrow (BM) harboring many different microenvironmental niches. LLPCs play an essential role in the humoral immune protection by maintaining constant high-affinity antibody levels against pathogens and their toxic products, independently of antigen presence.
So far, the in vitro long-term cultivation of BM LLPCs is challenging since they reveal a brief survival time ex vivo. Thus, this project aims to develop an in vitro model that mimics the physiological microenvironment of their niche in the BM and enables long-term cultivation of LLPCs.

METHODS:Our previously published data show that a three-dimensional (3D) model based on a hydroxyapatite-coated zirconium oxide-based ceramic could be used to maintain hematopoietic stem and progenitor cells for up to 8 weeks in their undifferentiated state (CD34+CD38-) when co-cultivated with BM mesenchymal stromal cells (MSCs) (Sieber et al. 2018). Based on this data, the model is adapted to establish a microenvironment with required cell-cell interactions and secreted survival factors to support the survival and maintenance of functional LLPCs in vitro.
Human plasma cells (PCs) (CD38+CD138+) are isolated via magnetic activated cell sorting (MACS) from femoral head after mechanical preparation and introduced into the scaffold mentioned above.

RESULTS:To show the survival capacity of the cultured PCs in the 3D scaffold, two strategies are pursued. On the one hand, the PCs are extracted from the scaffold again and viable PCs expressing CD38 and CD138 are quantified by flow cytometric analysis. On the other hand, their survival is shown indirectly by making use of their ability to secrete immunoglobulins. So, the detection of secreted antibodies by Bioplex shows the presence of viable and functional PCs.

DISCUSSION & CONCLUSIONS:By including further signaling factors, the in vitro PC survival can be even prolonged. Previously performed transcriptome analyses of primary MSCs are used to determine PC niche relevant soluble factors that are lacking in our culture system and are necessary to supplement to generate a more in vivo like microenvironment.
The established survival niche model could serve as a system to study niche interactions and will pave the way to establish disease models for diseases like multiple myeloma or autoimmunity to analyze changes in the microenvironment that promote the maintenance of pathogenic PCs. The better understanding of the survival mechanisms of pathogenic PCs could disclose new targets for specific therapies.

Keywords: In vitro microenvironments, Disease models

High-shape controlled liquefied capsules for tissue engineering strategies

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INTRODUCTION:Spherical encapsulation systems are the most widely used geometry for modular tissue engineering (TE) strategies. However, the fabrication of multi-shaped complex 3D structures is being proposed to mimic the complexity of native tissues, and thus promote a proper tissue remodeling. Here, we propose the development of Liquefied and Multilayered Capsules (LMC) at high rates and with defined sizes and shapes using the effect of discontinuous dewetting on a superhydrophobic-hydrophilic microarray. We have previously developed LMC↓ that solved diffusion issues as well as ensured physical support for anchorage-dependent cells.[1] LMC are composed by (i) a multilayered membrane that ensures permeability to essential molecules for cell survival; (ii) surface functionalized poly(ε-caprolactone) microparticles (μPCL) loaded into the liquified core acting as cell adhesion sites; and (iii) cells.

METHODS:Microgels with different geometries were obtained by slow-gelation of alginate containing a 2:1 ratio of CaCO3:glucono-δ-lactone (GDL). μPCL and adipose-derived stromal cells (ASCs) were added to the alginate solution. Afterwards, microgels were prepared by discontinuous dewetting on a slide with superhydrophobic surfaces patterned with wettable superhydrophilic domains. Following crosslinking, the slide was immersed in a NaCl/MES solution and microgels with different geometries (circles, squares and cylinders) were easily detached forming freestanding microgels encapsulating ASCs and μPCL. Subsequently, layer-by-layer was performed using poly(L-lysine), alginate, and chitosan as polyelectrolytes, in order to build the multilayered membrane around the microgels. Ultimately, the liquefied core was obtained by chelation with EDTA. LMC with defined shapes were cultured for 7 days.

RESULTS:The successful development of multi-shaped LMC encapsulating μPCL and ASCs was visualized by light microscopy. Live-dead assay shows that, after 7 days of culture, the majority of encapsulated ASCs remained viable. Fluorescence staining of F-actin filaments evidences the interaction and structural organization of the encapsulated cells with the μPCL inside the compartmentalized and controlled environment of LMC.

DISCUSSION & CONCLUSIONS:Accordingly, we have demonstrated a high-reproducible method to fabricate free-standing LMC with defined geometries and sizes. The developed LMC can potentially be explored as building blocks for bottom-up strategies, within the concept of modular TE. The idea is to use the well-established cell friendly environment provided by LMC, and create more close-to-native systems owning high heterogeneity, while providing multifunctional and adaptive inputs.

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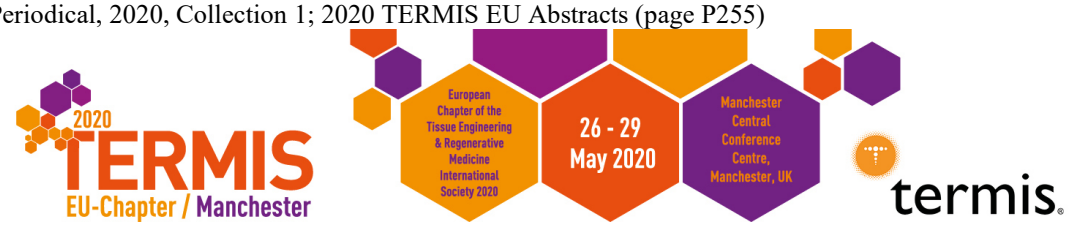
Keywords: Stem cell niche, Biomaterials



Development and Physiological Testing of Auxetic Scaffolds for Tissue Engineering

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INTRODUCTION:Some natural biological tissues display auxetic properties where upon stretching in the axial direction they also expand laterally, becoming thicker, which corresponds to a negative Poisson's ratio. A porous auxetic scaffold has the potential to mimic the properties of naturally auxetic tissue. Here, we investigate the mechanical properties of fabricated auxetic and composite scaffolds using 3D digital image correlation (DIC).

METHODS:Unconverted scaffolds were fabricated from polyurethane using a previously developed technique using thermomechanical triaxial compression [1]. Composite scaffolds were made using converted scaffolds which were filled with a thermoresponsive pNIPAM-Laponite ® hydrogel (1C10) (HG). The mechanical properties of the scaffolds were assessed by uniaxial tensile testing on a Cellscale univert and 3D DIC (LaVision Strainmaster) in physiological conditions. Smooth muscle cells were suspended in HG at 4x106 cells/ml at 37ºC. The hydrogel was absorbed into the scaffold and set by lowering the temperature below 32ºC. Scaffolds were cultured under static conditions for up to 6 weeks. Duplicate scaffolds were cultured under dynamic cyclic tensile load in a Cellscale MCT6 for 2 weeks. All samples were histologically processed to assess cell attachment, cytocompatibility, cell morphology and proliferation.

RESULTS:Auxetic scaffolds (converted) were successfully fabricated demonstrated by gaining a negative Poisson's ratio when compared to the unconverted control. Composite scaffolds were not auxetic. Smooth muscle cells cultured within the composite scaffold in static culture were suspended in the pores, supported by the hydrogel. Scaffolds cultured under cyclic load had a different macroscopic structure caused by the cyclic loading with fewer cells found within the scaffold.

DISCUSSION & CONCLUSIONS:3D DIC has been successfully used to measure the Poisson's ratio of a variety of scaffolds with both auxetic and conventional behaviour. The Auxetic scaffolds could be used to recapitulate the biophysical environment cells experience within auxetic tissue when under load. This holds potential for the creation of auxetic constructs for use within tissue engineering.

ACKNOWLEDGEMENTS:Acknowledgements:This work was supported by a grant from Sheffield Hallam University, Pioneer Healthcare and Sheffield Children's NHS Foundation Trust.

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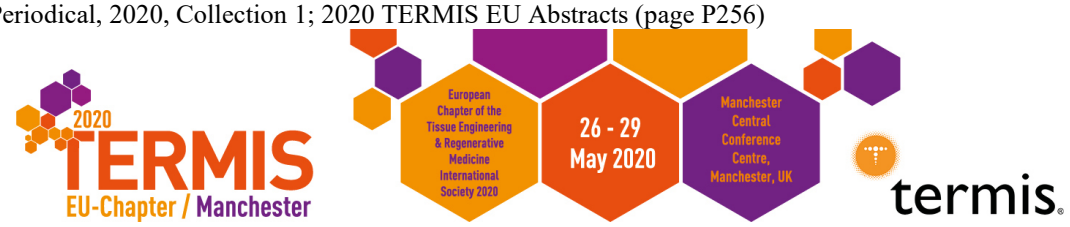
Keywords: Biomaterials, Hydrogels and injectable systems



Synergistically enhanced ex vivo expansion of hematopoietic stem/progenitor cells by cellular and mechanical cues

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INTRODUCTION:Hematopoietic stem/progenitor cell (HSPC) transplantation is used in the treatment of blood-related malignant and inherited diseases. Ex vivo expansion of HSPCs has been investigated to improve the clinical outcome of HSPC transplantation, particularly in cases with limited grafting size. In nature, HSPCs are located mainly in the bone marrow interact with a specific microenvironment referred to as stem cell niche, which regulates HSPC fate.

METHODS:Here, we co-cultured HSPCs with mesenchymal stem cells (MSCs) and divided the HSPCs into two fractions according to whether they came into adherent to MSCs or not. Additionally, we used hydrostatic pressure (HP) to mimic the physical conditions in vivo.

RESULTS:The expanded total nucleated cell (TNC) count for non-adherent cells was significantly higher than that of adherent cells. However, the adherent cells maintained the HSPC phenotype ($CD34^+$ and $CD34^+CD38^-$) to a greater degree than the non-adherent cells over the course of 4 days. The adherent cells also had superior clonogenic potential as assessed by colony-forming cell (CFC) and long-term culture-initiating cell (LTC-IC) assays. We also used a bioreactor to provide HP. We found that expanded cells from groups subjected to HP tended to have higher numbers of TNCs than did those from groups that were not subjected to HP. Interestingly, when HP was applied, the HSPC phenotype frequency and clonogenic potential were significantly higher than when HP was not applied. However, when HSPCs were cultured without MSCs, HP application had little effect on the maintenance of the HSPC phenotype or the clonogenic potential of expanded cells. To identify the cause of these synergistic effects, we harvested co-cultured MSCs under different culture conditions and assessed gene expression for HSPC niche markers (ANGPT1, ANGPT2, JAG1, OPN and RUNX2). As expected, we found that the expression levels of all markers were significantly higher in MSCs co-cultured with HSPCs compared with those with MSC alone.

DISCUSSION & CONCLUSIONS:Our study demonstrated that the direct contact between HPSCs and MSCs enhances the maintenance of an immature HSPC phenotype and function. Additionally, we showed that HP significantly influences the outcome of HSPC expansion and the maintenance of stemness. It is the first study which applies HP to the expansion of HSPCs. These results are expected to have an important impact on ex vivo expansion of HPSCs and eventually on the design of an efficient clinical-scale expansion system.

Acknowledgements:This work was supported by the National Research Foundation of Korea (NRF) Grant (NRF-2015M3A9B6073643).

Keywords: Microenvironment and niche engineering, Stem cell niche

Mimicking stem cell niches to study three dimensional migration of hematopoietic stem/progenitor cells

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INTRODUCTION:Hematopoietic stem/progenitor cells (HSPCs) have the property to return to the bone marrow, which is believed to be critical in situations such as HSPC transplantation. This property plays an important role in the stemness, viability, and proliferation of HSPCs, also. However, most in vitro models so far have not sufficiently simulated the complicate environment. Here, we proposed a three-dimensional experimental platform for the quantitative study of the migration of HSPCs.

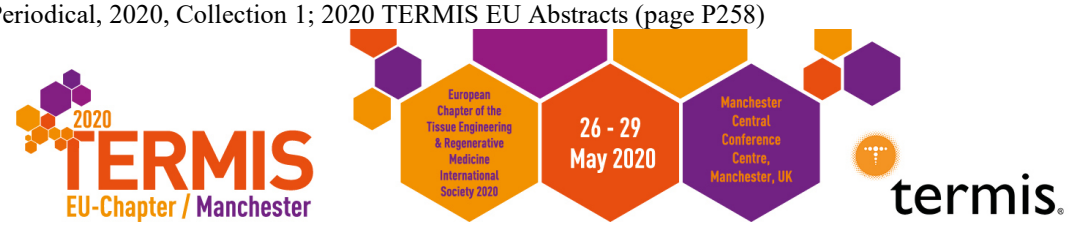
METHODS:After encapsulating osteoblasts (OBs) in alginate beads, we quantified the migration of HSPCs into the beads due to the physical environment using digital image processing. Intermittent hydrostatic pressure (IHP) was used to mimic the mechanical environment of human bone marrow without using any biochemical factors. IHP was applied using a bioreactor at a pressure of 20 kPa for 2 min, followed by no pressure for 4 min. The expression of stromal cellderived factor 1 (SDF-1) under IHP was measured.

RESULTS:The results showed that the presence of OBs in the hydrogel scaffold initiate the movement of HSPCs.Furthermore, the IHP promotes the migration of HSPCs, even without the addition of any biochemical factors, and the results were confirmed by measuring SDF-1 levels.

DISCUSSION & CONCLUSIONS:This study proposed a simple in vitro BM biomimetic system that can be used to study HSPC migration. The ability of IHP to simulate the physical conditions in the BM related to the movement of HSPCs was confirmed by measuring SDF-1 expression. The IHP stimulation affected OBs to induce increase of SDF-1 expression. We believe this suggested three-dimensional experimental platform consisting of a simulated in vivo physical environment and encapsulated OBs should contribute to in vitro migration studies used to investigate the effects of other external factors.

Acknowledgements:This work was supported by the National Research Foundation of Korea (NRF) Grant (NRF-2015M3A9B6073643).

Keywords: Microenvironment and niche engineering, Stem cells – general



Mesenchymal stromal/stem cells from synovium as potential osteoarthritis concause

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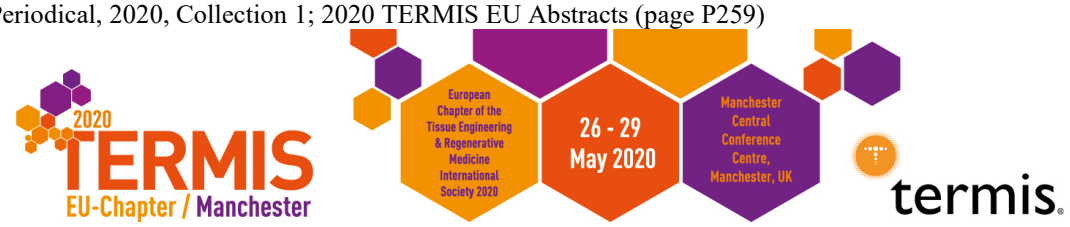
INTRODUCTION:Mesenchymal stromal cells recruited in the synovium (Synovium-Derived Stromal Cells; SDSCs) are generally considered for their regenerative potential in cartilage lesions [1,2]. These cells, however, could be involved in Osteoarthritis (OA) pathophysiology. Herein, we assessed this occurrence.

METHODS:Histological, immunohistochemical, ultrastructural and flow cytometry analyses were initially used to compare SDSCs isolated from healthy and OA subjects in terms of phenotype, morphology and differentiation potential. Moreover, SDSCs' capability to activate normal Peripheral Blood Mononuclear Cells (PBMCs) was investigated with a co-cultural approach, integrating morphological analysis with qRT-PCR and functional resorbing assays. Synovia was harvested during surgery for total knee arthroplasty in eight OA subjects and in two healthy subjects undergoing leg amputation. In accordance with the Local Ethical Committee guidelines and with the 1964 Helsinki declaration an informed consent was obtained from all individual participants included in the study.

RESULTS:Our data demonstrated that both SDSC populations were similar in terms of differentiation capability toward cartilagineous and osteoblastic lineages, as well as in the formation of multinucleated cells from PBMCs. However, the osteoclast-like cells generated by healthy-SDSCs via trans-well co-cultures were inactive, whilst OA-derived SDSCs had the capability to resorbs dentin slices. Moreover, the presence of a further stromal cell type with a peculiar ultrastructure called telocytes (TCs) was more evident in cultures obtained from OA subjects that suggested their potential involvement in OA.

DISCUSSION & CONCLUSIONS:Based on our in vitro study, it can be argued that SDSCs have a double effect on osteoclasts depending on the microenvironment. Indeed, we determined that SDSCs were capable to stimulate osteoclastogenesis by means of soluble factors, but the multinucleated cells generated by healthy-SDSCs were dormant. In contrast, OA-derived SDSCs had greater effectiveness in stimulating active osteoclastogenesis. Our findings may open an interesting opportunity for the development of new approach for OA treatment, that considers the multifaced capability of MSCs in relation to the specific environment
[2] doi:10.1186/scrt501

Keywords: Multipotent (mesenchymal) stem cells, Cartilage / joint and arthritic conditions



Clarifying interplay between dental MSC and biomimetic material under dynamical conditions for bone tissue engineering

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INTRODUCTION:Massive amounts of cell are needed for tissue engineering, which often requires culture on 3D scaffolds under dynamic conditions. Dynamic cultures are expected to improve cells viability and proliferation rate, as compared to 2D static culture [1]. However, cells from distinct types and/or sources may eventually respond differently to external stimulation and may be incompatible to culture under mechanical stress.

METHODS:In this work, mesenchymal stem/stromal cells (MSCs) isolated from human dental follicle and pulp tissue were shown to express stem cell markers. A multicompartment holder adaptable to spinner flasks was used for dynamic culture (50 rpm) of both dental MSCs types after pre-seeding in 3D porous collagen/nanohydroxyapatite-phosphoserine cryogel scaffolds [2]. Standard static culture conditions were used as control. Cellular engraftment and survival were evaluated in vivo by CBA nude mice (8 week-old, i3S animal house, Portugal) after implantation of a cell-loaded scaffolds. After 8 weeks scaffolds were explanted and the resulting bone-like tissues were evaluated by immunohistochemistry and histology analysis.

RESULTS:Culture under dynamic conditions promoted follicle MSCs proliferation, while improving cellular spatial distribution within the scaffold. The modified biocomposite promoted osteogenic differentiation, as suggested by increased alkaline phosphatase (ALP) activity, osteopontin expression and matrix mineralization (Von Kossa). However, dental pulp MSCs showed higher alkaline phosphatase (ALP) activity and higher osteocalcin gene expression, when under static conditions. After 3 weeks in vitro culture and 4 weeks in vivo of osteogenic induction by the biomimetic scaffold (Coll/nanoHA_OPS5), dental follicle MSC's were able to differentiate into an osteoblastic phenotype and produce mineralized ECM (osteopontin).

DISCUSSION & CONCLUSIONS:Human dental follicle and pulp MSCs were successfully isolated, characterized and osteogenic differentiated. Bone-like tissue growth into the implanted cell-loaded scaffolds after 8 weeks (ectopic ossification) could established a proof-of-concept on the feasibility and reproducibility of dental derived MSC's in the bone regenerative processes. Overall, this study explored the use of an innovative device for dynamic culture of cell-laden 3D scaffolds, showing that MSCs behavior is influenced by culture conditions. This suggests that cells should be carefully selected according to the application.

Acknowledgements:Supported by project NORTE-01-0145-FEDER-000012, Norte Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL2020 Partnership Agreement, through the European Regional Development Fund (ERDF). Also by Portuguese funds through FCT/MCTES in the framework of the project UID/BIM/04293/2019 and C.L. Salgado contract (DL57/2016/CP1360 /CT0001).

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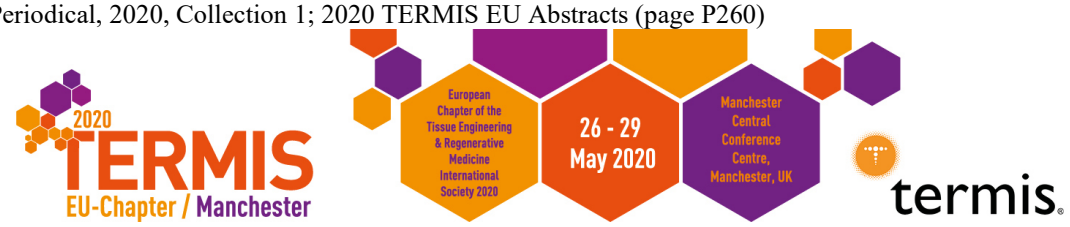
Keywords: Multipotent (mesenchymal) stem cells, Differentiation



Using Nanoflares to Isolate Skeletal Stem Cells from Human Bone Marrow

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INTRODUCTION:Skeletal stem cells (SSCs) can differentiate along the osteogenic, adipogenic and chondrogenic lineages, thus offer significant interest as potential clinical therapies. Human bone marrow derived stromal cells (HBMSCs) contain the SSCs, however, current methods to isolate skeletal stem cells (SSCs) from human tissues have remained difficult in the absence of a single specific marker. Thus, understanding SSC fate, immuno-phenotype and simple selection criteria are limiting factors in the widespread clinical application of these cells. While a range of cell surface markers can enrich for SSCs, none of the proposed markers alone can isolate a homogenous population with the ability to form bone, cartilage, and adipose tissue in humans.

METHODS:Previous studies have attempted to use oligonucleotide-coated gold nanoparticles (Smartflares) that required exposure of the particles to cells for extended culture times (~18 hours). We have developed a new innovative methodology to use these nanoparticles with a 1-hour contact time to identify cells in human bone marrow displaying specific mRNA signatures to isolate and enrich for the SSCs.

RESULTS:Exposure of HBMSCs to nanoparticles for 18 hours reduced cell viability in cell lines. Using a 1-hour incubation was sufficient to detect specific mRNA signatures in cells evidenced by flow cytometry to enable sorting of “positive” and “negative” cell fractions, without affecting cell viability. Critically, using a range of mRNA target sequences, we have enriched SSCs 2000-fold.

DISCUSSION & CONCLUSIONS:We demonstrate, for the first time, the ability to use oligonucleotide-coated gold nanoparticles to detect and enrich, 2000-fold, human SSCs based on their mRNA signature. In addition, we demonstrate that the nanoparticles did not allow quantification of mRNA. The developed methodology allows the rapid (4 hours) isolation of uncultured SSCs from human bone marrow samples in previously unavailable quantities, with tremendous potential and implications for regenerative medicine for an increasing aging demographic.

ACKNOWLEDGEMENTS:This work is funded by BBSRC.

Keywords: Bone and bone disorders (osteoporosis etc), Stem cells – general

Dynamic Oxygen Pre-conditioning of Mesenchymal Stem Cells to Enhance Therapeutic Angiogenesis – Applications to Tissue Engineering and Regenerative Medicine

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INTRODUCTION:Mesenchymal stem cells (MSCs) have the most established track record for translational use, but how to fine-tune their cellular function and behaviour for specific in vivo use is still not fully understood. Hypoxia is one of the main driving forces for the upregulation of angiogenic factors in MSCs.[1] Our aim is to determine the exact in vitro oxygenation conditions through dynamic pre-conditioning in 2D and 3D settings, which can help further potentiate the angiogenic pathway for therapeutic in vivo applications.

METHODS:Bone marrow (BM) and umbilical cord (UC) derived MSCs were pre-conditioned in normoxia (21% O2) and physiological hypoxia (5% and 1% O2) and subsequently seeded into Type I collagen hydrogels. These cell-loaded 3D hydrogels were subsequently subjected to three different oxygen conditions (1%, 5% and 21%). Media from different time points were collected and analysed for VEGF levels and RNA from cells were extracted for qPCR of HIF1a and stemness markers. Viability and proliferative capacity were also determined.

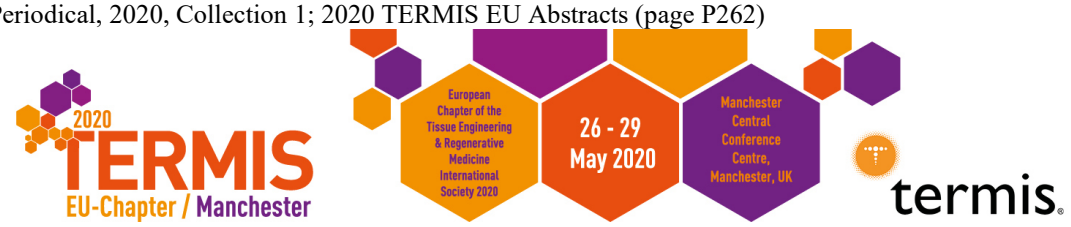
RESULTS:Cells pre-conditioned in 2D in hypoxia (5% O2) prior to being seeded did not exhibit increased VEGF production when compared to cells pre-conditioned in normoxic (21% O2) conditions. Cell viability between cells grown in 21% and 5% were not significantly different, but proliferative ability decreased when cells were cultured in 1% O2. Normoxic pre-conditioned cells produced more angiogenic factors and HIF1a gene upregulation when subsequently placed in 3D hypoxic conditions (5% and 1%), than if they were pre-conditioned in hypoxia and then cultured in 3D hypoxia. Hypoxia enabled maintenance of stemness markers.

DISCUSSION & CONCLUSIONS:These preliminary results offer an indication to how cells behave in 2D and 3D settings in dynamically changing oxygen environments, particularly when transferred from 2D normoxic to 3D hypoxic conditions. This offers an insight into in vivo cell behaviour after therapeutic transplantation. The results also address the effects of chronic hypoxia on MSCs and the effects of changes in oxidative stress on the cells and their angiogenic signalling. Further work is required to determine if such enhancement in angiogenic factor production would translate into improved neoangiogenesis in vivo. Such research into optimising the stem cell niche for therapeutic purposes will have huge impact on future cell therapy uses in regenerative medicine, such as wound healing and tissue engineering.

ACKNOWLEDGEMENTS:This study is funded by the MRC and Rosetrees Trust

REFERENCES:1. Pugh et al. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med 9, 677–684 (2003)

Keywords: Cell therapy, Vascular systems / vascularisation and heart



Characterisation of an immune-primed subset of mesenchymal stromal cells in vitro and in vivo

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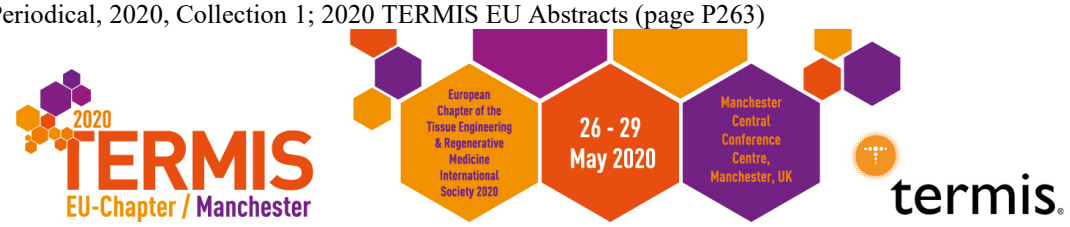
INTRODUCTION:The advancement of therapies using mesenchymal stromal cells (MSCs) is a priority in regenerative medicine. Clonal human MSC subpopulations have varied differentiation capacities, and we identified a biomarker (CD317) that allows discrimination of differentiation-competent MSCs (CD317-) from non-differentiating MSCs (CD317+). Here, we determined the potential impact of the CD317+ MSC subpopulation on inflammatory disease processes and regenerative therapies.

METHODS:Clonal lines and primary CD317- and CD317+ MSCs were used with QPCR and ELISAs to determine gene/protein expression including interferon-γ scores. Flow cytometry measured ex vivo T-cell responses in co-culture with MSCs; in vivo impact of MSC treatments were determined in a mouse peritonitis model, whilst tissue regeneration was evaluated in immunocompromised mice using subcutaneously implanted MSC-loaded hydroxyapatite scaffolds.

RESULTS:CD317+ MSCs comprised 0.01-47.41% of primary populations (mean 9.78%±2.33,n=31), which had increased IFN-γ scores compared to CD317- MSCs (0.987±0.320 vs 0.020±0.005); increased IFN-γ scores were also observed in CD317+ versus CD317- clonal MSC lines (1.430±0.541 vs 0.040±0.036). Compared to CD317- MSCs, CD317+ MSCs also expressed higher levels of genes associated with chemokine signalling, leukocyte migration and adhesion (CXCL10, CXCL11, CX3CL1, SSA2, SSA4 and ICAM-1). CD317+ MSCs showed a reduced ability to limit T-cell proliferative cycles compared with CD317- MSCs in both CD317+ clonal lines (4.98±0.48) versus CD317- lines (2.33±0.59) from controls (5.96±0.72); and primary CD317+ MSCs (4.47±0.23) versus CD317- MSCs (4.00±0.04) from controls (5.26±0.26). Primary CD317+ MSCs also failed to reduce an index of overall proliferative capacity (6.89±0.36) versus CD317- (4.29±0.36) from controls (7.44±0.81). CD317+ clonal MSCs increased T-cell polarisation towards pro-inflammatory Th1 cells compared to CD317- co-cultures (15.81±1.17% vs 7.45±0.45%). An increase in regulatory T cells was also observed with CD317+ clonal lines compared to CD317- MSCs (10.21±2.14 vs 7.01±1.37%); an effect replicated in primary MSC co-cultures. In vivo, CD317- and CD317+ MSCs suppressed leukocyte recruitment (2.08±0.42x10^6; 3.55±1.54x10^6) compared to zymosan-induced peritonitis (9.69±1.89x10^6). In contrast, the development of antigen-specific CD8a+ cytotoxic T cells was suppressed in spleens of CD317+ MSC-treated mice (2.08±0.50%) but not CD317- MSC-treated (5.95±0.74%) compared to animals without any treatment (5.33±0.78%), suggesting a complex interaction between immune cells in response to stromal subtypes.
In vivo implantation of CD317- MSC-loaded scaffolds demonstrated enhanced tissue generation, with evidence of vessel formation by 3 weeks post-implantation, whereas CD317+ MSCs had weak regenerative capacity.

DISCUSSION & CONCLUSIONS:The presence of subpopulations of MSCs that do not contribute to tissue formation and elicit enhanced immunomodulation could detrimentally affect tissue regeneration and offset clinical outcomes in therapeutic applications.

Keywords: Stem cells - general, Immunity / immunomodulation / macrophage



Effect of obesity on adipose stem cells and their capacity for immunomodulation using genetically matched obese and lean donors

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INTRODUCTION:During the past decade obesity has become a major worldwide health problem. Obesity is strongly associated with chronic low-grade inflammation with a number of metabolic diseases including insulin resistance, type 2 diabetes, atherosclerosis and several cancers with high economical and societal consequences. Cell-based therapies are a novel approach to treat medical conditions that have limited or no effective therapeutic options. Particularly, adipose tissue (AT) -derived multipotent cells known as adipose stromal/stem cells (ASCs) are promising candidates for diverse clinical applications because the excellent proliferation and differentiation capacity and ability for immunomodulation. This work investigates the effects of obesity on ASC characteristics and especially on ASC-mediated immunomodulation.

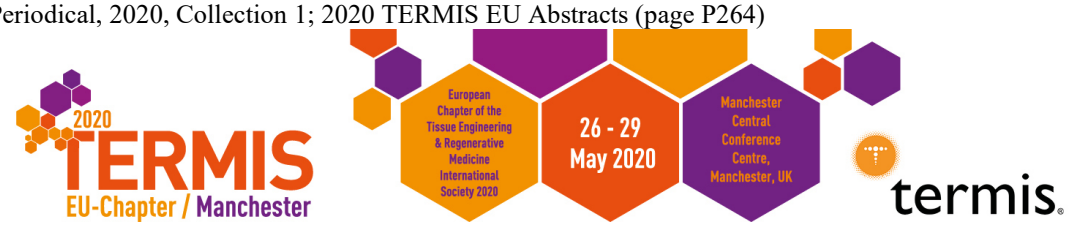
METHODS:We have collected a unique patient material of obese donors who undergo weight loss treatment. We obtained AT samples from bariatric surgery before weight loss, and a second AT sample after weight loss, which offers us valuable material to study the effects of obesity while genetic influences are controlled. In addition to isolation of ASCs, we prepared histological sections from both obese and lean AT samples. The immunosuppressive mechanisms of actions of ASCs were investigated through co-cultures of ASCs and human immune cells, specifically umbilical cord derived naïve T cells and peripheral blood derived macrophages. The differentiation of naïve T cells into specific T cells subtypes and polarization of macrophages into pro- and anti-inflammatory directions were studied in co-culture with obese and lean ASCs. The immunophenotype of obese vs. lean ASCs, as well as polarization of monocytes and T cells was analyzed using flow cytometry. Moreover, the inflammatory status of obese and lean AT was characterized from histological sections.

RESULTS:Our preliminary data and hypothesis suggest that ASCs derived from lean AT will induce more anti-inflammatory macrophages and regulatory T cells in co-culture as compared with ASCs from obese AT of the same donor. Moreover, histological evaluation of obese AT revealed infiltration of macrophages as well as altered cell morphology as compared with lean AT of the same donor.

DISCUSSION & CONCLUSIONS:This work provides important knowledge on obesity-induced alterations on ASCs and their capacity for immunomodulation. ASCs have an evident role in the regulation of AT inflammation and metabolism, but the mechanisms are not fully understood. This work will increase our understanding about cell-level mechanisms behind the development of metabolic diseases and in long-term will offer novel therapeutic insights for metabolic diseases.

Acknowledgements:This work was supported by Academy of Finland (grant number 311084).

Keywords: Immunity / immunomodulation / macrophage, Cell therapy



How to create an GMP-grade artificial urinary conduit using tissue engineering methods: Impact of the cell culture reagents on the product quality

Kamil SZELISKI, Monika Anna BUHL, Daria BALCERCZYK, Tomasz KLOSKOWSKI, Marta RASMUS, Arkadiusz JUNDZIłł, Tomasz DREWA, Marta POKRYWCZYńSKA
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INTRODUCTION:Tissue engineered neo-conduit for urinary diversion is an alternative for currently used method with usage of intestine wall after complete bladder removal. However optimization of Good Manufacturing Practice (GMP) compliant procedures is required before possible clinical application. The aim of the study was to compare influence of different GMP-grade media on the growth, and properties of human Adipose Derived Stromal Cells (hADSC) on artificial conduit in the previously developed dynamic culture method.

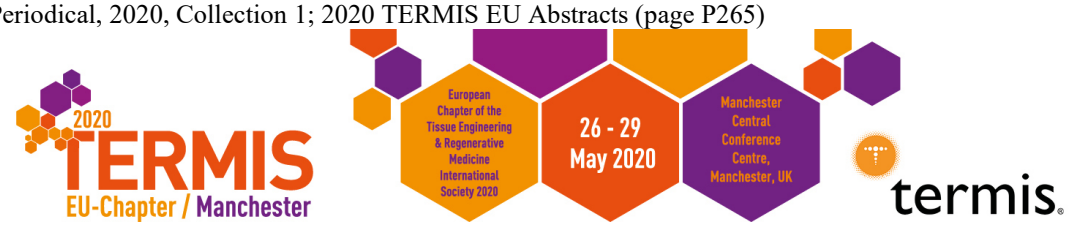
METHODS:hADSC cells were seeded on artificial conduit and cultured in dynamic conditions in bioreactor for 7 days. Two previously evaluated serum free, GMP-grade media were used: MEM-alpha + 10% human platelet lysate, and DMEM + 10% human platelet lysate. Cells phenotype, apoptosis level and cell cycle were evaluated with flow cytometry directly after ending a 7-days dynamic culture and 3 hours later.

RESULTS:Cells were successfully cultured on artificial conduit in both media, with dynamic culture method. Differences in CD105 levels were observed between dynamically and statically cultured cells, as well as between cells detached from grafts cultured in compared media. Small to no differences in level of other positive (CD44,CD73,CD90) and negative markers (CD11b, CD34, CD45, HLA-DR) were observed. No significant differences were observed in cell cycle and apoptosis level in both media.

DISCUSSION & CONCLUSIONS:Both media present promising, comparable results. The results indicate that better GMP-grade medium for culture of artificial urinary conduit seeded with hADSC is DMEM with 10% human platelet lysate, as the changes in cells phenotype were smaller compared to second tested medium.

Acknowledgements:The present work was supported by the National Center for Research and Development (NCBR) in Poland under Agreement no. STRATEGMED1/235368/8/NCBR/2014 (Smart AUCI Project) within the Strategic Programme STRATEGMED “Prevention practices and treatment of civilization diseases.”

Keywords: Cell therapy



Myocardin-related transcription factor A (MRTF-A) regulates the fate decision of human adipose stem cells into osteoblasts or adipocytes

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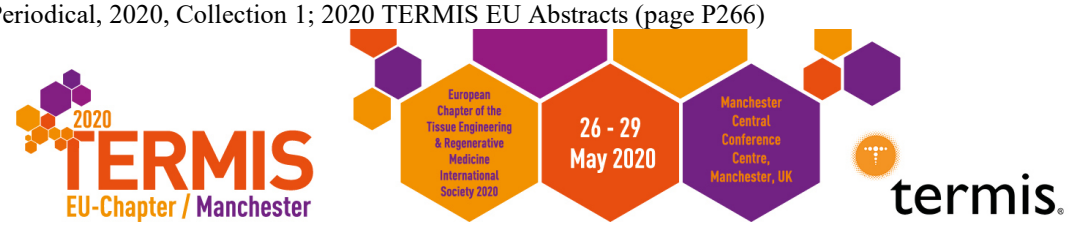
INTRODUCTION:The actin cytoskeleton of a cell is continuously modified to allow dynamic cell functions such as migration, proliferation and differentiation. These modifications are accomplished by actin turnover through the reversible polymerization of actin monomers into filaments. The ratio of the monomers and filaments in turn regulate transcription of actin related genes through myocardin-related transcription factor A (MRTF-A) and its co-regulator serum response factor (SRF). While previous studies have shown the importance of cell shape and regulation of actin cytoskeleton in the differentiation fate of stem cells, the role of these transcription factors remain understudied. Thus, our aim was to elucidate the significance of MRTF-A mediated transcription in the regulation of human adipose stem cell (hASC) differentiation.

METHODS:To study the role of MRTF-A signaling in the differentiation process, we cultured hASC in adipogenic and osteogenic media supplemented with inhibitor molecules CCG-1423 or CCG-100602 that have been shown to block the expression of MRTF-A/SRF mediated genes. Adipogenic differentiation was analyzed with Oil Red O staining while activity of an early osteogenic marker alkaline phosphatase, mineralization of extracellular matrix (ECM) and collagen type I production were studied to evaluate the osteogenic differentiation of the inhibitor treated cells.

RESULTS:We found that MRTF-A inhibition led to lowered activity of alkaline phosphatase and reduced the collagen type I export into the ECM of hASC. Additionally, the inhibitors significantly decreased the matrix mineralization of hASC indicating reduced osteogenic differentiation. On the other hand, inhibition of MRTF-A signaling led to increased quantity of Oil Red O stained lipid droplets.

DISCUSSION & CONCLUSIONS:Our results suggest that MRTF-A signaling is capable of regulating the switch between osteogenesis and adipogenesis in the hASC differentiation. These results provide a new interesting target for controlling the stem cell differentiation in future treatments and tissue engineering applications.

Keywords: Differentiation,



Comparison of Multipotential Stromal Cells from subchondral bone and synovial fluid in Knee Osteoarthritis

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INTRODUCTION:There is limited information on the role of multipotential stromal cells (MSCs) in musculoskeletal disease pathogenesis in humans. Osteoarthritis (OA) severity is often associated with an abnormal load distribution on the medial compartment of the knee. As the MSCs from the different niches of the joint are unable to restore the diseased tissues in late disease, the aim of this study was to determine differences in MSC from subchondral bone (SB-MSC) vs synovial fluid (SF-MSC) in OA knee. Also, we investigated the profile of miRNA cargo carried in extracellular vesicles (EVs) secreted by medial OA SB-MSC (OAMSC-EVs) compared with those secreted by healthy MSC (HMSC-EVs).

METHODS:OA SF-MSCs were obtained from synovial fluid during total knee replacement [1] and SB-MSCs were extracted after digestion of the femoral condyles from these patients [2]. Gene expression, phenotypical characterization, motility assay and growth rate were analysed in both cell types. MSCs from healthy donors were isolated from bone marrow aspirate and culture expanded for EV isolation. EVs from all MSC samples were isolated by differential ultracentrifugation [3] and characterized according to MISEV guidelines before miRNA profiling using NanoString technology.

RESULTS:OA SB-MSC and SF-MSC showed comparable trilineage potency, growth rate, surface phenotypes and motility, in vitro. Seven genes were upregulated in SF-MSC compared with SB-MSC: THBS4 (63.03-fold, p=0.0003), MMP9 (18.97-fold, p=0.0050), ADAMTS5 (8.59-fold, p=0.0003), IGFBP3 (7.12-fold, p=0.0007), VEGFC (5.42-fold, p=0.006), TIMP3 (4.79-fold, p=0.003) and SERPINE1 (2.59-fold, p=0.0056). Three genes were upregulated in SB-MSC compared to SF-MSC: IGF2 (428.33-fold, p=0.0020), IBSP (122.06-fold, p=0.0068), RUNX2 (5.41-fold, p=0.0007). miRNA profiling showed 46 miRNA differentially expressed between OAMSC-EVs and HMSC-EVs.

DISCUSSION & CONCLUSIONS:Despite the location of the MSCs in different niches of the joint, no major differences were found SB-MSC and SF-MSC in terms of in vitro functionality and phenotype. However, SB-MSC presented upregulation of genes implicated in osteogenic homeostasis and SF-MSC upregulation of catabolic and anabolic genes implicated in matrix remodelling. Also, there were differences in miRNA between OA and healthy MSC-EVs, for which the target genes and implicated pathways need further investigation.

Acknowledgements:CSR is beneficiary of a postdoctoral fellowship from Xunta de Galicia.

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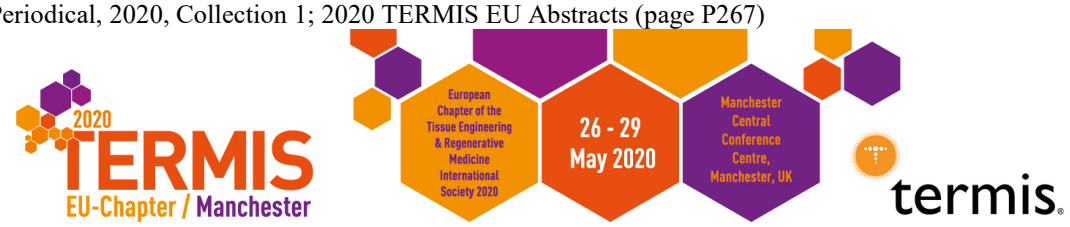
Keywords: Cartilage / joint and arthritic conditions, Extracellular vesicles



A donor-matched comparison of adipose and bone marrow-derived mesenchymal stem cells reveals enhanced anti-inflammatory properties in adipose stem cells: Potential implications for IVD regeneration

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INTRODUCTION:Degeneration of the intervertebral disc (IVD) is a leading cause of lower back pain, and a significant clinical problem. Inflammation mediated by Interleukin-1 beta (IL-1β) and tumour-necrosis factor alpha (TNF-α) drives IVD degeneration through promoting a phenotypic switch in the resident nucleus pulposus (NP) cells towards a more catabolic state, resulting in extracellular matrix degradation. Bone marrow mesenchymal stem cells (MSCs) produce bioactive factors that modulate local tissue microenvironments and their anti-inflammatory potential has been shown in numerous disease models, offering a potential therapy for IVD degeneration. In a clinical setting, adipose-derived stem cells (ASCs) might represent an alternative and perhaps more appealing cell source due to their faster proliferation rate and reported differentiation to NP-like cells [1]. However, their anti-inflammatory properties remain poorly understood.

METHODS:Donor-matched human ASCs and MSCs were cultured in monolayer and exposed to an inflammatory stimulus of 10ng/ml IL-1β and/or 50ng/ml TNF-α, or indirectly co-cultured with degenerate human NP cells. The anti-inflammatory properties of ASCs/MSCs were assessed by qPCR and western blotting of whole cell lysates. RESULTS:We demonstrate that stimulating ASCs or MSCs with IL-1β and/or TNF-α elicits a strong anti-inflammatory response with increased expression of IL-1 receptor antagonist (IL-1Ra), cyclooxygenase-2 (COX-2) and the tissue protective protein tumour-necrosis factor stimulated gene-6 (TSG-6). ASCs produced significantly higher levels of IL-1Ra and TSG-6 than their matched MSCs at both gene and protein levels, indicating that ASCs are potentially a more potent anti-inflammatory cell type. This anti-inflammatory response was also observed upon co-culture with degenerate NP cells without exogenous cytokine. Signalling analyses suggested this difference between cell types could be mediated through differences in the activation of the inflammation-associated transcription factors nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT-3).

DISCUSSION & CONCLUSIONS:To our knowledge, this study is the first direct comparison of the anti-inflammatory properties of donor-matched human adipose and bone marrow-derived mesenchymal stem cells. These data indicate that ASCs may possess more potent anti-inflammatory properties than matched MSCs, and so may be the more useful cell source in the development of future therapies for IVD degeneration.

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Keywords: Intervertebral disc / spine and their disorders, Cell therapy

Impact of adipose tissue storage conditions on the adipose derived stromal cell isolation yield

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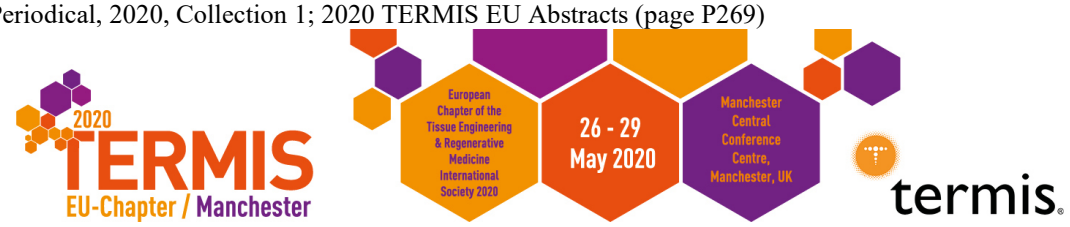
INTRODUCTION:Adipose tissue is a highly specialized tissue that has many different functions in the human body. It is also an excellent source of stem cells used in the regenerative medicine and tissue engineering. In the present paper, we evaluated the impact of human adipose tissue preservation conditions on the number of isolated human adipose derived stromal cells (hADSCs) and the number of cells in primary culture.

METHODS:Subcutaneous adipose tissue was harvested from patients' abdominal area during a liposuction (n=10). Adipose tissue samples were stored in different solutions: Biolasol Solution (B), culture medium MEM Alpha supplemented with platelet lysate and antibiotics (M); physiological saline with 0.15%lidocaine + epinephrine 5 µg/ml (1:200 000)(S), at the temperature of 4°C for 3,6,12 and 24 hours. Freshly harvested adipose tissue was used as a control sample. Adipose tissue was digested in collagenase NB6 for 20 min. The isolated cells were seeded at 20.000 cells/cm2 in tissue culture flasks. Cells were grown to 80% confluence. Immunophenotype of hADSCs was analyzed with the flow cytometry.

RESULTS:In average, the highest number of cells were isolated from the adipose tissue directly after harvesting (624424.84±195052.02), significantly lower number of cells were isolated after 6 hours (441708.33±174367.14;$p<0.05$), 12 hours (432027.91±182321.18;$p<0.05$) and 24 hours (379226.68±159653.20;$p<0.05$) of tissue storage.
The highest number of cells were isolated from the adipose tissue stored in M (490899.87±185790,07) as well as in S (449367.54±164163.19), significantly lower number of cells were isolated from the adipose tissue stored in B (376767.72±176349.86;$p<0.05$;$p<0.01$).
The number of viable cells from the primary culture was significantly lower for the adipose tissue samples after 24 hours of storage (30844.64±15076.57) in comparison to the number of the cells isolated from freshly harvested adipose tissue (62142.86±35079.93;$p<0.05$); and adipose tissue stored for 3 or 6 hours (55078.95±25826.06;$p<0.05$;50159.38±30728.24;$p<0.05$, respectively).
The lowest number of viable cells in the primary culture was observed when B was used for tissue storage (29234.78±17637.01;$p<0.05$), and was significantly lower compared to the number of cells isolated from tissue stored in S
(51271.74±17637.01;$p<0.05$) or M (52300.60±26351.31;$p<0.05$)
.
DISCUSSION & CONCLUSIONS:Adipose tissue subjected to cell isolation should be processed within the time not exceeding 3 hours after harvesting. The Biolasol solution should not be used for adipose tissue storing before isolation of hADSCs.

ACKNOWLEDGEMENTS:The present work was supported by the National Center for Research and Development (NCBR) in Poland under Agreement no. STRATEGMED1/235368/8/NCBR/2014 (Smart AUCI Project) within the Strategic Programme STRATEGMED "Prevention practices and treatment of civilization diseases."

Keywords: Stem cells – general



Molecular aspects of adipose-derived stem cells in long-term culture

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INTRODUCTION:Adipose derived stromal cells (ADSCs) isolated from adipose tissue have become the leading type of mesenchymal stromal cell used for tissue engineering application. Data about ADSCs characteristic during long term culture are sparse. That is why it is very important to show how long ADSCs retain their regenerative properties and determine the maximum passage after which ADSCs can be used for clinical application without signs of senesce.

METHODS:ADSCs were isolated from porcine adipose tissue and cultured in standard conditions at 37°C with 5% CO2 atmosphere and 98% humidity. ADSCs from the 2nd (P2, control group), 6th (P6) and 10th (P10) passage were placed in RNA later solution and stored at - 80°C. Gene expression of tested cells was determined by RT-PCR according to RT2 Profiler PCR Array Handbook using RT2 Profiler PCR Array Pig Aging on LightCycler 480. The data analysis was performed at GeneGlobe Data Analysis.

RESULTS:The molecular analysis revealed a different senescence gene expression profile in subsequent ADSCs passages. Comparing P6 cells and P2 cells we observed over-expression of CD14, CA4, CALB1, COL3A1 genes and under-expression of CFH gene. The most significant changes were shown between P10 cells and P2 cells. Over-expression of CA4, C1S, C3AR1, CD14, CX3CL1, CXCL16, TLR4, FOXO1, RNF144B, TXNIP, ARID1A, EP300, EP300, EP300, EP300, COL3A1, LOC100628185, NDUFB11, SIRT3 and SIRT3 was observed. The under-expressed genes were LOC102164975, LOC102164975, S100A9 and LMNB1.

DISCUSSION & CONCLUSIONS:The study showed that the best time point up to which ADSCs should be used for further in vitro and in vivo experiments is the 6th passage.

ACKNOWLEDGEMENTS:The present work was supported by the National Center for Research and Development (NCBR) in Poland under Agreement no STRATEGMED1/235368/8/NCBR/2014 (Smart AUCI Project) within the Strategic Programme STRATEGMED “Prevention practices and treatment of civilization diseases”.

Keywords: Stem cells – general

Evaluation of the Vascular Potential of Human Umbilical Cord Mesenchymal Stem Cells for the Generation of Palate and Oral Mucosa Substitutes

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INTRODUCTION:Current cleft palate treatments are subjected to several drawbacks and complications. The onset of tissue engineering may allow a regenerative approach to patients with cleft palate and oral mucosa diseases. However, one of the limitation of the use of orofacial tissues generated by tissue engineering is the lack of a blood vessel network in the bioengineered tissue. In this work, we have analyzed the vascularization potential of Human Umbilical Cord Wharton's Jelly Stem Cells (HWJSC) to determine their potential use in vascularization of palate and oral mucosa substitutes.

METHODS:Human umbilical cords were obtained, and 4 types of samples were generated: Wharton's jelly histological samples fixed in formalin (UC-S), primary HWJSC bi-dimensional cultures (UC-2D), three-dimensional HWJSC spheroids (UC-Sph) and HWJSC membranes (UC-Mem). All samples were processed histologically and the endothelial markers CD31, VEGF and von Willebrand factor (vWf) were detected by immunofluorescence. In addition, samples were analysed by scanning electron microscopy (SEM) after fixation in 2.5% buffered glutaraldehyde.

RESULTS:First, we found that the human umbilical cord Wharton's jelly is able to express in situ significant amounts of the CD31, VEGF and vWf endothelial markers, although important differences were detected among the different zones of the umbilical cord, with perivascular and intravascular zones showing the highest expression. In addition, we found that the number of cells and the extracellular matrix density differed among the different zones of the human umbilical cord. Then, we found that UC-2D cultures showed lower expression of these endothelial markers, whereas UC-Sph and UC-Mem showed increased endothelial markers expression.

DISCUSSION & CONCLUSIONS:Our results support the use of HWJSC for the induction of a vascular network in oral mucosa and palate tissues generated by tissue engineering. The specific potential of cells in each area of the umbilical cord may vary, with the intervascular zone being the most suitable cell source to generate vascularized palate constructs by tissue engineering. In addition, the loss of vascular cell potential in bidimensional cultures suggest that three-dimensional cultures techniques should be used for an efficient vascularization of artificial palate and oral mucosa.

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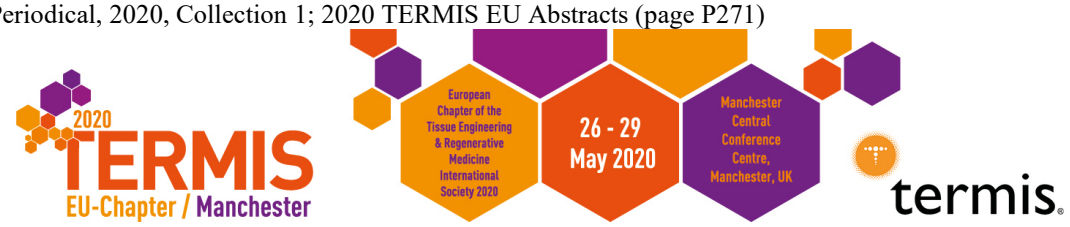
Keywords: Biologics and growth factors, Multipotent (mesenchymal) stem cells



Cell Distribution in a Three-Dimensional Scaffold via microCT Analysis

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INTRODUCTION:Uniform cell distribution is crucial to enable cell-cell interaction, nutrient distribution, uniform ECM deposition and uniform tissue build-up within the scaffold. Underpopulated areas can indicate poor cell suspension, nutrient deprivation and problematic scaffold design. Most studies use cryo-sectioning to assess cell distribution[1],[2]. However, for larger engineered tissues this would result in hundreds of slides, time consuming analysis and the destruction of the scaffold. Furthermore, some materials are hard to section due to their low melting point. In this study we explored microCT as an alternative method to assess cell distribution throughout electrospun scaffolds designed for tendon tissue engineering

METHODS:Electrospun PCL scaffolds were seeded with human hamstring cells and left for 1 hour to attach. Seeding methods included Upright - filaments aligned with gravity, Flat - aligned perpendicular to gravity and Rocked - perpendicular to gravity and set on a rocking plate. Cells were fixed in 2.5% glutaraldehyde and stained with Osmium Tetraoxide (OsO4). Samples were scanned in a microCT together with a no-cell control. The Fiji 3D-Object counter was used to visualize cells above the threshold. Interpolated results were binned and plotted in a frequency distribution. Further analysis included SEM and Elemental x-ray analysis.

RESULTS:Results showed a more even cell distribution for the Rocked sample, low cell numbers in the middle portion of the flat sample, more cells at the bottom and very few cells at the point of seeding on the Upright sample. The same can be seen in the frequency distribution graphs, the volume render and the heat map. SEM analysis showed cells were easier to locate at the ends of each sample compared to the middle and appeared in clusters. The no-cell control showed no cells. Elemental x-ray analysis confirmed peaks around the electromagnetic emission spectrum of osmium on cell-seeded scaffolds, compared to no peak on the no-cell control.

DISCUSSION & CONCLUSIONS:The methods used are straight forward, coherent and visualize cell distribution throughout a scaffold without the need of cryo-sectioning. Complementary x-ray analysis on the no-cell control leads to the conclusion that positive signal detected in the µCT scans are in fact cells. It was concluded that rocking the sample immediately after seeding leads to better cell distribution throughout the scaffold.

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Keywords: Imaging - advanced, Biomaterials

Tissue waste-derived tendon inductive bioactive factors: new perspectives to foster tendon healing and regeneration

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INTRODUCTION:The co-culture between stem cells and specific tissue explants/cell lines has been proposed to induce stem cell differentiation [1]. The purpose is to reproduce in culture the cocktails of molecules driving physiologically tissue repairing. The present research aimed to exploit such a mechanism in promoting tenogenesis [2] in amniotic epithelial stem cells (AECs) taking advantage of ovine foetal Tendon (fT) explants.

METHODS:AECs and fT were collected from slaughter house and cultured as follow: AEC: cells alone, fT: foetal Tendons alone; fT+AEC: co-culture. Conditioned Media (CM) were collected from fT (CMfT) and fT+AEC (CMfT+AEC) cultures every two days, diluted 1:1 and used to test on AEC the tendon inductive effect by analysing: epithelial mesenchymal transition-EMT (Twist, Snail, Zeb and Vimentin) or tendon-related genes (Scxb, Thbs4, Tnmd, Col3 and Col1) and tendon specific proteins (Tenomodulin and Collagen type 1).

RESULTS:The CMs regulated differently EMT and tendon differentiation: CMfT induced a significantly upregulation of Twist and Vimentin ($p<0.05$) whereas it was less effective in inducing tenogenesis. In order to induce the over expressions of early (Scxb) and late tendon genes (Thbs4, Tnmd, and Col3) AEC had to be exposed to fT or to CMfT+AEC. This tendon-inductive influence was also confirmed by Col1 and Tnmd proteins deposition in the extracellular matrix of tendon like 3D structures that AEC differentiated after 14 days of culture.

DISCUSSION & CONCLUSIONS:Tenogenesis can be induced collecting soluble factors generated through the in vitro active dialogue between AEC and fT. Indeed, exclusively CMfT+AEC induced AEC to move towards a late tendon phenotype. In conclusion, CM may be proposed as a new waste tissue product to make available low-cost tendon-inductive bioactive compounds supporting a challenging field such as R&I in regenerative medicine applied to tendon repair.

Acknowledgements:Research funded by Italian Ministry of Instruction, University and Research under the national project PON research and innovation 2014-2020.

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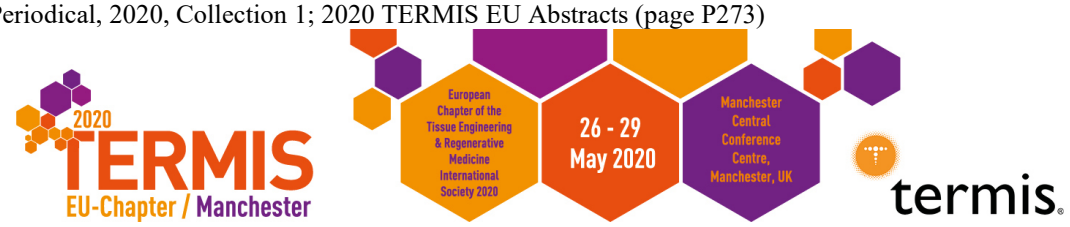
Keywords: Differentiation, Stem cells – general



human Bone Marrow Mesenchymal Stem Cells cultivated in a 3D microenvironment with controlled delivery of human Growth Differentiation Factor 5: a study on their tenogenic commitment

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INTRODUCTION:Tissue engineering strategies to optimize a high predictive in vitro models for stem cells differentiation and commitment versus tenogenic phenotype can improve knowledge and protocols for tendon healing and regeneration. Indeed, tendinopathies represent major medical issues associated with physical activity and age-related degeneration. In this context, a tridimensional (3D) and totally biodegradable and bioresorbable scaffold arranged to provide both biochemical and mechanical inputs represent an efficient highly predictive in vitro system for both in vitro and in vivo studies.

METHODS:A biomimetic 3D microenvironment was designed for human Bone Marrow Mesenchymal Stem Cells (hBM-MSCs) tenogenic commitment. This 3D tissue-like scaffold is formed by a hyaluronate braided band merged with fibrin hydrogel to secure hBM-MSCs and poly-lactic-co-glycolic acid (PLGA) nanocarriers for the controlled delivery of human Growth Differentiation Factor 5 (hGDF-5). The elastic band allows the system strain by means of a culture bioreactor system [1, 2, 3]. Immunofluorescence (IF) and quantitative real time polymerase chain reaction (qRT-PCR) analysis were used to understand the cells behavior.

RESULTS:The proposed combined approach favors the organization of a 3D tissue-like structure promoting a remarkable arrangement of the cells within the fibrin environment and the neo-extracellular matrix, reflecting into hBM-MSCs commitment towards a tenogenic phenotype.

DISCUSSION & CONCLUSIONS:The described bioengineered scaffold system combined effect of the mechanical and biochemical stimuli on hBM-MSCs providing in situ strain and growth factor delivery. The data described gave evidence in support of the 3D system, its interaction with cells, and open present potential for its use as a predictive in vitro model for tendon regeneration study.

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Keywords: Stem cells - general, Differentiation

A hybrid approach to engineer the tendon synovial sheath for prevention of post-operative adhesions

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INTRODUCTION:Clinical treatments for tendon lacerations can be compromised by adhesion formation[1] due to tendon synovial sheath disruption and aberrant healing. Hence, there is the need to develop novel anti-adhesion systems capable of allowing tendons to glide. One of the most promising approaches relies on the introduction of a biomembrane that acts as a physical barrier for adhesion-forming cells whilst regenerating the tendon synovial sheath[2]. Here, we propose a novel hybrid approach that combines electrospinning and 3D bioprinting techniques to produce a bilayer biomembrane for restoration of tendon synovial sheath integrity and prevention of post-operative adhesions.

METHODS:Polymeric meshes were prepared by electrospinning, using a 10% w/v solution of poly(ε-caprolactone) (PCL) (Mn=50,000) dissolved in 1,1,1,3,3,3,-hexafluoro-2-propanol with 1ml/h flow rate, 20kV voltage and 20cm needle-collector distance. Samples were imaged using Scanning Electron Microscopy and mechanically tested under tension. Migration studies were performed by allowing human dermal fibroblasts (HDFs) to migrate for 24h through the PCL mesh in a double chamber system. HIG-82s (synoviocytes) were encapsulated in Alpha4 self-assembling peptide hydrogel (SAPH) and cell-laden constructs were printed using a 3D Discovery bioprinter. Cell viability and metabolic activity were evaluated over 4 weeks via LIVE/DEAD and AlamarBlue assays, respectively. Production of hyaluronic acid (HA) by encapsulated cells was assessed using Alcian Blue staining (Scott method) and immunocytochemistry. Pull-out tests were performed on a porcine ex vivo model to investigate the membrane's frictional properties.

RESULTS:Results show that a nanofibrous mesh (Mean=0.254µm) is produced with mechanical properties that can withstand the forces generated in the tendon during motion. The mesh prevents the infiltration of HDFs in vitro due to its small pores (<3µm). 3D bioprinting allows for accurate spatial distribution of the hydrogel phase without affecting synoviocyte viability and proliferation. Moreover, Alpha4 SAPH provides a substrate that, mimicking the extracellular matrix of the native tissue, stimulates cells to produce HA. Ex vivo pull-out tests show that the final product possesses anti-adhesive properties.

DISCUSSION & CONCLUSIONS:To prevent post-operative tendon adhesions, a bilayer biomembrane to be wrapped around the tendon is proposed. The biomembrane acts as physical barrier and has the potential to restore the native content of HA for long-term lubrication. Future steps will include animal work to evaluate the product's effectiveness in vivo.

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Keywords: 3D printing and bioprinting, Hydrogels and injectable systems

A Multi-scale approach to reconstruct a bioartificial muscle-tendon system

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INTRODUCTION:Tissue Engineering (TE) is a promising approach to repair muscles or tendons when natural healing fails in cases of trauma or disease. These alterations may lead to progressive muscle injury, inflammation, necrosis and cause abnormalities at the myotendinous junction (MTJ). In addition, TE could be very useful in providing insights into the mechanisms of formation of these tissues and more specifically on their interfaces. The challenge is thus the design of biohybrid scaffolds able to mimic the architecture of native tissue as well as their mechanical properties. Scaffolds for the TE of skeletal muscle should mirror the parallel alignment of native myofibrils and enhance the myogenic differentiation. Biochemical, and physical stresses may play a supportive role in inducing cellular alignment in such engineered tissues. The aim of this study was to investigate the synergistic effects of topographical, mechanical and electrical stimulations cues on skeletal muscle and tendon cells' development and the formation of the myotendinous junction.

METHODS:We developed a composite scaffold using electrospun fibers of poly(ε-caprolactone) (PCL) covered with gold nanoparticles (Np), on which we micropatterned Poly(ethylene glycol) hydrogel by photolithography. The cultures of C2C12 myoblasts cell line for the muscle-like tissue and rats' MSC for the tendon-like tissue were carried out over a period of 7 and 12 days in static condition or under mechanical and/or electrical stimulation. Cell behavior was followed through the quantification of cell viability, proliferation, alignment and differentiation, assessed by fluorescent staining, MTT assay.

RESULTS:We showed that micropatterning the hygrogel on the PCL/Np scaffold provided geometrical cues that significantly influenced the alignment and differentiation process of C2C12 cells. When associated with electrical and/or mechanical stimulations, an increase in myogenic differentiation and myotube alignment was displayed. Moreover, the direct co-culture of C2C12/MSCs allowed the formation of a merge zone at the contact of both cells types, as well as the expression of oriented collagen fibers in the tendon-like area.

DISCUSSION & CONCLUSIONS:We studied how an appropriate scaffold design and external stimuli enhanced the myogenic differentiation and cells alignment. In the long run, we propose have found an effective approach to characterize the first steps of myotendinous junction formation.

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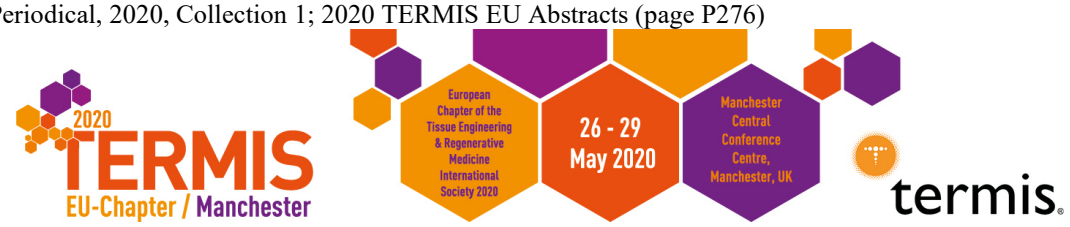
Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc), Biomaterials



Fabrication and characterization of an elastomeric 3D system with a macroscopic soft porous scaffold for biohybrid actuation

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INTRODUCTION:In the last years, skeletal muscle cells (SMCs) have been exploited as force generators for constructing bidimensional biohybrid actuators. Only a few examples deal with three-dimensional devices, but the compactness of the final constructs hampers cell survival when scaling up in dimensions (>150 µm-thick)[1]. Here we present a polyurethane soft porous scaffold assembled in a system based on a thin stretchable membrane isolating the scaffold from the external environment, and on elastomeric links for force transmission.

METHODS:The polyurethane scaffolds were supplied by Tensive S.r.l. (cylindrical shape, diameter: 9 mm, length: 10 mm, purified in low-boiling point organic solvent)[2]. Scaffold permeability was measured as in [3]. Uniaxial compression tests were performed using a ±10 N load cell (Instron 5965, 2 mm/min). Young's moduli were obtained considering strain up to 10%. Cyclic compressions (5%, 1500 cycles) were performed at 1 Hz (softening degree: ratio between the maximum stress at 1st cycle and at 1500th cycle). For the system assembly (5.5-cm length), the scaffold was encapsulated in a polydimethylsiloxane shell (1:20 ratio curing agent:monomer, 100µm-thick) and physically crosslinked to two hollow tendon-like polydimethylsiloxane links (1:10) at the extremities.

RESULTS:We fabricated a system resembling a tendon-muscle-tendon anatomical unit. The central scaffold presented interconnected cavities with a diameter of 651.35 ± 20.29 µm, a porosity of 93% (n=2) and a permeability of 1.77e-9 ± 3e-10 m² (n=4, mean ± SD). The scaffold Young's modulus was 5.9 ± 1.01 kPa, and with the polydimethylsiloxane shell, it increased to 12.59 ± 1.38 kPa (n=4, mean ± SD, $p \leq 0.05$). The median softening degree of the encapsulated scaffold was 0.87% (n=3). Preliminary in vitro tests verified C2C12 cell line adhesion on fibronectin-coated scaffold.

DISCUSSION & CONCLUSIONS:The system presents a scaffold with features sustaining cell viability (porosity >80% for nutrient distribution) and fostering SMC differentiation (muscle-like stiffness)[3]. Furthermore, it presented a low softening degree, which is a relevant parameter when assessing scaffold resistance to fatigue when repeatedly compressed (as during SMC contraction). The shell had a negligible influence on the scaffold stiffness and permitted the preservation of an isolated environment, scalable in dimensions, in which cells can be seeded by perfusion through the polydimethylsiloxane links. The links can be used for the mechanical stimulation of differentiating SMCs, and for transmitting the cell-generated force of the contracting actuator.

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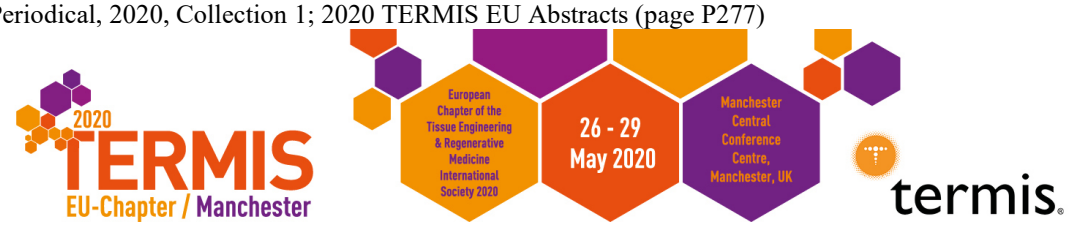
Keywords: Interfaces - engineered, Bioreactors



Myogenic commitment of human mesenchymal stem cells

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INTRODUCTION:Skeletal muscles heal themselves through a regeneration process. During the process immune system cells are involved. Indeed, during the first stage of the regeneration process, mast cells and neutrophils activate, making the microenvironment pro-inflammatory. During the second stage, T lymphocytes activate themselves and macrophages are recruited at injury site. In particular, macrophages have a pro-inflammatory phenotype, called M1, they secrete pro-inflammatory cytokines, as IL-1, TNF-α, present high expression levels of iNOS releasing NO to promote necrosis debris clearance. At the last stage of the process, macrophages turn their phenotype from pro- inflammatory M1 to anti-inflammatory M2. Anti-inflammatory cytokines release, such as IL-10 and IL-12, and T-reg lymphocytes activation promote the healing process successfully. So, both macrophage phenotypes are required for muscle healing. Their lack or their continuous expression of M1 phenotype causes an impaired healing process.

METHODS:In vitro 3D bioengineered systems were fabricated using collagen or fibrin and cultured in dynamic condition using perfusion bioreactor. The 3D systems allowed the co-culture of human Bone Marrow-Mesenchymal Stem cells (hBM-MSC) and human Macrophages(hM) polarized by cytokines stimulation. Myogenic commitment was obtained using growth factors embedded within the scaffold system.

RESULTS:hBM-MSC gene expression of myogenic transcription factors as Myf5, MyoG, MyoD and MRF4 were used to detect cells commitment as well as hM gene expression such as CD68, CD80/CD86 (M1>M2), CD163 (M2 marker) and CD206 (M2>M1 marker) were used to understand macrophage phenotype during the co-culture. Fibrin scaffold and perfusion conditions seemed the best in vitro environment for the proposed study.

DISCUSSION & CONCLUSIONS:The 3D in vitro system developed successfully induced myogenic commitment of hBM-MSC; the macrophages co-cultivation effect was also investigated. The 3D system developed can be used as highly predictive model to study the macrophage effect on skeletal muscle regeneration. Acknowledgements:The authors acknowledge Athena srl for funding a PhD grant (Cycle XXXV) and a research project entitled: "Skeletal muscle regeneration modulated by inflammation: an in vitro study on a 3D bioengineered system".

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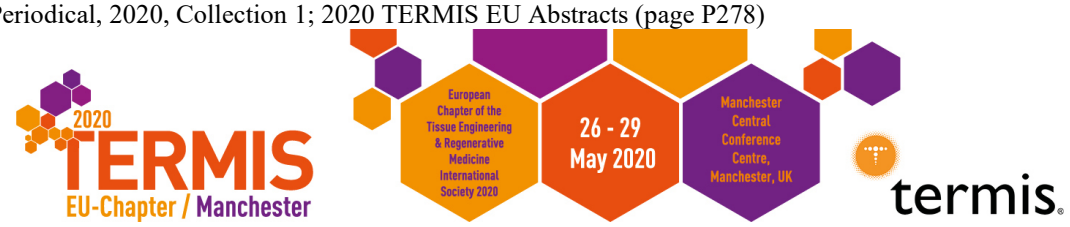
Keywords: Multipotent (mesenchymal) stem cells, Bioreactors



Developing advanced nanofibrous moieties via electrospinning for tissue engineering

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INTRODUCTION:Electrospinning is an exciting fiber production method that has emerged as a promising approach for tackling many of the current challenges faced in the field of tissue engineering. By pushing through its limits, nanofibrous scaffolds produced via electrospinning present excellent biocompatibility, while the capability of tuning the materials' properties and achieving suitable micro-/nanostructures can prompt favorable cellular activities that guide tissue regeneration. In this work, synthetic and natural polymer-based material were integrated to develop nanofibrous constructs capable of tuning physiochemical properties by varying the forms of Poly(glycerol sebacate) [1] within the polymer blends. By the inclusion of Silk fibroin, the desired biocompatibility of the scaffolds was obtained, while the incorporation of Poly(caprolactone) improved the mechanical integrity of the electrospun mats.

METHODS:In this work:
• A nozzle-free electrospinning device was developed, comprised of a rotating cylinder electrode submerged within a Teflon pool, and a biased rotating collector electrode under constant supply of nitrogen gas. A high potential difference of 60 kV was applied between the two rotating electrodes (+30/-30 kV), resulting in the formation of multiple Taylor cones on the rotating electrode surface immersed in the solution bath, from which jets stretched to form fibers in an upward motion.
• A variation of the conventional protocol for the extraction of silk fibroin from Bombyx mori cocoons (molecular cut-off technique), based on ethanol precipitation, was exploited. [2,3]
• The effect of the pre-polymerized (pPGS) and PGS on the composite's chemical behavior, mechanical stability, wettability and biocompatibility, was assessed.

RESULTS:We observed that:
1. The fiber morphology and mechanical behavior are dependent on the polymerization time of the PGS.
2. FTIR, XPS, DSC, and TGA asserted the presence of all three polymers in the composite structure.
3. The presence of SF and PCL retarded the degradation rate of PGS within the scaffolds.
4. The wettability was manipulated based on the proportion of PGS and pPGS within the scaffold.
5. In vitro studies with fibroblasts presented good viability, attachment, and proliferation.

DISCUSSION & CONCLUSIONS:The nozzle-free electrospinning technology developed, along with the straightforward SF extraction method employed, enables PCL-backbone Silk fibroin: PGS fibrous scaffolds to be promising candidates as substitute constructs for skin regeneration.

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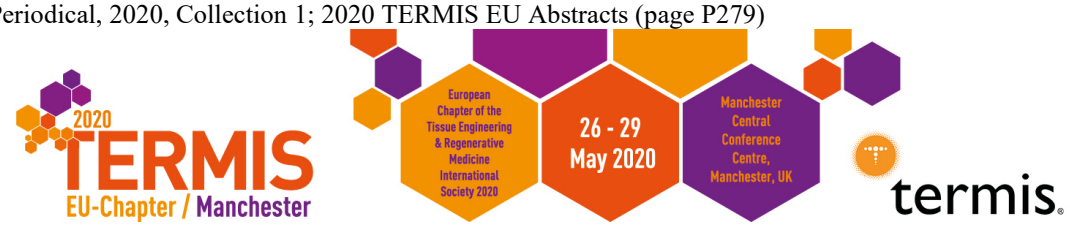
Keywords: Biomaterials, Nanomaterials (inc graphene)



Mild photothermal cancer therapy with graphene-based materials and their drug conjugates

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INTRODUCTION:Oncological malignancies are the second leading cause of death globally, raising the need for novel treatment strategies. Photothermal therapy (PTT) may be applied as an effective non-invasive alternative treatment. Near-infrared (NIR) light energy can induce hyperthermia (39-47°C), inducing higher nanoparticle/drug uptake due to increased membrane permeability and tumor cell apoptosis. Graphene-based materials (GBM) present strong radiation absorption and possess large surface area, holding potential for synergistic biologic and drug release hyperthermia triggered effects.1 Herein, GBM and GBM loaded with 5-fluorouracil (5-FU), an anti-cancer drug, are proposed as platforms for cancer PTT.

METHODS:Nano-sized graphene oxide (GOn) was produced through the modified Hummer's method2 followed by ultrasonication through a custom-built industrial grade system, to assure the achievement of reproducible large-scale batches of nano-sized GBM. Following a one-step procedure, GOn was thermally reduced and functionalized with poly(ethylene) glycol (PEG) moieties to obtain stable aqueous dispersions (rGOn-PEG).3 GOn and rGOn-PEG (0.25 mg/mL) were mixed with 5-FU at a drug concentration varying between 0.25-5 mg/mL. GBM aqueous dispersions were irradiated with a LED source of 812.8±29.9nm (150 mW/cm2) and temperature recorded using a thermocouple. The effect of GBM and NIR irradiation was evaluated by resazurin cell viability assay using a human skin carcinoma cell line (A431 cells, ATCC).

RESULTS:GOn was obtained with mean lateral dimensions of 248 nm, as determined by TEM. GOn and rGOn-PEG dispersions showed colloidal stability with zeta potential values around -25.6±0.8 mV and -10.2±0.3 mV (pH=7), respectively. 5-FU was successfully loaded by simple molecular physiosorption on GOn and rGOn-PEG, with loading capacity being of 5.8±0.8 mg 5-FU/mg GOn and 3.6±1.2 mg 5-FU/mg rGOn-PEG. NIR irradiation increased rGOn-PEG temperature to 47ºC after 30 min, which is within temperature ranges of hyperthermia. rGOn-PEG in combination with NIR reduced A431 cells viability, in opposition to irradiated GOn or rGOn-PEG alone.

DISCUSSION & CONCLUSIONS:This study opens new avenues for the development of GBM-based platforms for drug delivery and PTT of cancer.

Acknowledgements:This work was financed by FEDER - Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020 - Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through FCT/MCTES in the framework of the projects POCI-01-0145-FEDER-031143; POCI-01-0145-FEDER-006939; POCI-01-0145-FEDER-007274; PTDC/BTM-MAT/31143/2017, and Unidade de Investigação UID/EQU/00511/2019 - Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia – LEPABE.

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Keywords: Cancer, Drug delivery

Evaluating cellular behaviour under low oxygen to optimise the design of nerve repair constructs

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INTRODUCTION:Recently, scientific research in peripheral nerve injury treatment has focused on tissue engineered scaffolds that combine therapeutic cells and biomaterials, as they can provide mechanical support and a growth permissive environment to modulate regeneration[1–2]. One of the major determinants of the regenerative success of these constructs is the local microenvironment, which may exhibit low levels of oxygen[3]. However, experimental studies into these effects are scarce. Here, we aim to perform a variety of targeted experiments to further our understanding of the behaviour of therapeutic cells in engineered tissues under physiologically relevant conditions.

METHODS:Differentiated, clinically relevant neural stem cells (CTX0E03, ReNeuron Ltd, UK) were embedded in thin, stabilised collagen constructs to represent the implanted tissue structure2. A range of cell densities and physiologically relevant oxygen concentrations were used, as well as standard atmospheric oxygen. The impact of these culture conditions on cell survival, proliferation, vascular endothelial growth factor (VEGF) release as well as oxygen and glucose consumption was determined.

RESULTS:There were density- and oxygen-dependent changes in cell viability, oxygen and glucose consumption as well as growth factor release. For instance, compared to normal oxygen levels there was a 40% reduction in the metabolic activity of cells at 1% O2. These observations were used for parameterisation of a mathematical model to simulate the interactions between therapeutic cells, oxygen and growth factors.

DISCUSSION & CONCLUSIONS:Recapitulating the local oxygen microenvironment of the peripheral nerve injury site in vitro could allow us to make more informed predictions about the overall effectiveness and regenerative potential of cellular nerve repair constructs. Moreover, using a combined framework of experimental work and mathematical modelling could help us optimise the design of engineered neural tissue.

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Keywords: In vitro microenvironments, Stem cells – general

Gallic acid-based dendrimers inhibit Aβ42 amyloidogenesis and its associated cytotoxicity

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INTRODUCTION:The cleavage of the amyloid precursor protein (promoted by gamma-secretase) leads to the release and accumulation of amyloid-beta42 (Ab42) in the brain of Alzheimer's Disease (AD) patients.1 Its supramolecular assembly cause the formation of different higher-order species, from which the oligomeric forms are reported to be the most cytotoxic. These Ab42 oligomers present a dynamic character (i.e. capacity to assemble/disassemble into different structures); the capacity to interfere with the activity of different biomolecules; and the ability to spread throughout the brain, causing ultimately, cell death.2 The remodelling of their supramolecular structure is the key-step to reduce the presence of these cytotoxic oligomers. It has been described that gallic acid (GA) can interfere and interact (π-π stacking and H-bonding) with the hydrophobic residues of Ab42, promoting the remodelling of its supramolecular structure.

METHODS:We synthesized GA-terminated dendrimers that present an increasing number of GA units at their surface: 2G0-GaOH (2 gallates); 2G1-GaOH (6 gallates); and 3G1-GaOH (9 gallates). Their ability to inhibit the aggregation of Ab42 was evaluated by ThT, CD, AFM and MD simulations. SH-SY5Y cellular response was assessed by MTS, Live/Dead, immunostaining and BioAFM.

RESULTS:All dendrimers are able to remodel the Ab42 supramolecular assemblies through the reduction of their beta-sheet content and inhibiting the formation of fibrils/oligomeric species (monitored by CD, ThT and AFM). Moreover, MD simulations showed that the dendrimers interact preferentially with the Glu and Ala residues of Ab42, in a way directly proportional to the number of GA repeating units.
At the cellular level, the results of metabolic activity and live/dead assays show that 2G1-GaOH and 3G1-GaOH have the capacity to maintain SH-SY5Y cell viability. Additionally, both dendrimers reduced the deposition of Ab42 assemblies on cells (BioAFM and immunostaining), being the number of GA units determinant to produce this outcome.

DISCUSSION & CONCLUSIONS:We successfully developed GA-terminated dendrimers (2G1-GaOH and 3G1-GaOH) with the ability to modulate the supramolecular assembly of Ab42 and capacity to regulate its cytotoxicity. The remodelling is based on the formation of Ab42 off-pathway non-cytotoxic aggregates with a lower beta-sheet content. Our results show that the number of GA units at the dendrimers surface are key to eliminate the toxicity of Ab42 assemblies in the context of AD.

Acknowledgements:We acknowledge the EC for financial support: FORECAST - 668983 and THE DISCOVERIES CTR – 739572; ARA acknowledges Norte2020, NORTE-08-5369-FSE-000037 (PhD grant).

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Keywords: Nervous system (brain-central-peripheral / disorders), Nanomaterials (inc graphene)

Using Electrospun Scaffolds as a Novel Model for Urogynaecological Nerve Damage- a Pilot Study

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INTRODUCTION:Peripheral nerve damage during pregnancy and labour can cause a range of life-affecting medical issues, including stress incontinence in up to 36% of first deliveries [1, 2]. Currently there is no gold standard treatment, with patients often waiting to see if symptoms improve over time [1]. Many of the mechanisms behind this nerve damage and whether regeneration/repair of the nerve can follow are not fully understood. The aim of this research was to determine if electrospun scaffolds could be used to develop a novel model to further investigate urogynaecological nerve damage.

METHODS:A range of polyurethane electrospun scaffolds was UV-Ozone sterilised and cell-seeded with human neurons (HN) and Schwann cells (HSC): HN; HSC; HN+HSC. Seeding densities of 50,000 cells/type/sample were used for 4, 7, 14, 28 days.
Assessed substrate parameters included: inter-fibre separation (IFS); fibre diameter (FD); surface roughness (SR); void fraction (VF); fibre orientation (FO); stiffness (YM). Cell-scaffold behaviour was examined for cellular growth, marker expression, process length/involvement/function and myelination potential (n=4 per sample/condition).
ANOVA statistical tests, with Tukey post hoc analysis, were performed to determine correlative relationships for cell-scaffold behaviour.

RESULTS:Key aspects of neural cell behaviour and functionality were directly induced by separate and ranging scaffold properties. HN functionality, including axonal growth and alignment, was significantly affected by scaffold parameters in the horizontal plane (e.g. process length by FD, $P<0.001$ and 1/SR, $P=0.003$). HSC behaviour, including myelination potential, related to properties in the vertical scaffold plane (e.g. VF, $P=0.044$, for cell orientation). These relationships were seen in single and co-cultures.

DISCUSSION & CONCLUSIONS:Neural cell interaction, behaviour and function were significantly affected by these separate and ranging properties. Correlating the cellular effects to the underlying substrate parameters allowed individual causes of negative cell responses and repair behaviour to be identified. This suggested an ability to mimic natural neural regeneration and damage processes, through manipulation of distinct scaffold properties. Further translating this information into the clinical and anatomical equivalent situations/structures, through this novel model, can provide understanding of urogynaecological damage and potential treatment options.

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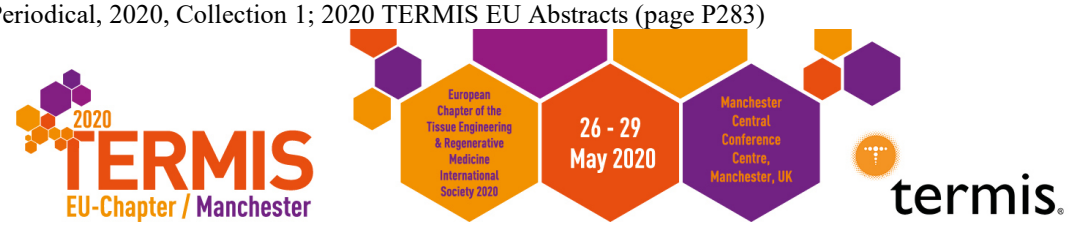
Keywords: Trauma / surgery and rehabilitation, Fibre technology



Generation of tissue scaffolds composed of aligned electrospun fibres of interpenetrating polymer networks of silk fibroin and PEDOT:PSS for peripheral nerve regeneration

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INTRODUCTION:Over 1 million people worldwide suffer from peripheral nerve injury (PNI) [1]. Electrical stimulation (ES) can be used in therapeutic purposes for the relief of nerve pain, and can also activate neurite outgrowth of neuronal cells in vitro [2, 3]. Therefore, the integration of ES with an electrically conductive nerve conduit may accomplish the regeneration of fully functional nerves. Here, we describe the development of electroactive composites of silk fibroin (SF) and poly(3,4-ethylenedioxythiophene)-polystyrene sulfonate (PEDOT:PSS) as interpenetrating polymer networks (IPNs) of sub-micron fibres as novel peripheral nerve tissue scaffolds.

METHODS:Non-woven mats of sub-micron fibres were fabricated based on double layers of electrospinning SF (randomly aligned - base and aligned - top). The electrospun SF materials were treated with 80% ethanol (EtOH) and then soaked in a solution of EDOT monomer, PSS and initiators for 3 days. The concentration ratio between EDOT and PSS (α) was varied from 1.3 to 3.3, to form IPNs. The physical and mechanical properties were characterized by scanning electron microscopy (SEM), hard X-ray photoelectron microscopy (HAXPES), and tensile testing. The optimal conditions of IPNs fibres were coated with laminin, their cytotoxicity and biocompatibility with the neuron-like cell line (NG108-15) were tested for 7 days.

RESULTS:The fibre diameter of electrospun materials was 190 ± 50 nm and no significant difference was observed after modification with PEDOT:PSS. The PEDOT:PSS modified fibres were turned dark blue. There was a significantly higher atomic percentage of Sulphur (S) in the IPNs compared to SF fibres. The stain at break and toughness of IPN materials were decreased when $\alpha = 2.8$ and 3.3. Additionally, cell metabolic activity and DNA concentration of NG108-15 cultured on IPN materials were steadily increased from day 1 to day 7.

DISCUSSION & CONCLUSIONS:The sub-micron aligned SF fibres are fragile in the dry state but flexible when hydrated due to plasticization of the SF by water. HAXPES and cytotoxicity results suggest that the electroactive SF:PEDOT:PSS IPNs are biocompatible. Moreover, the electroactive fibres can support neural cell proliferation and also neurite outgrowth when coated with laminin. The electrical conductivity of the fibres and its relation to external ES will be studied in depth in the future.

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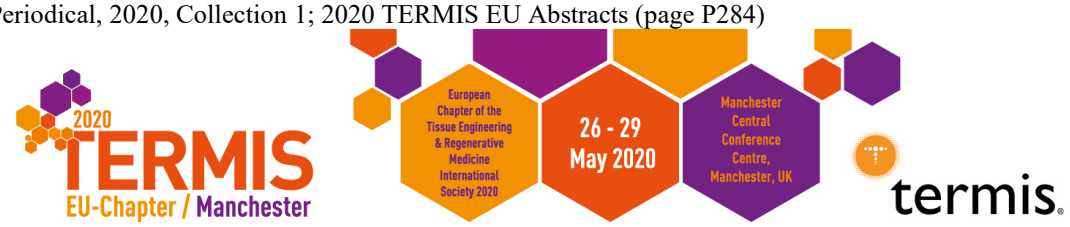
Keywords: Biomaterials, Nervous system (brain-central-peripheral / disorders)



Self-assembling peptide hydrogels for therapeutic use in brain tissue engineering and regeneration after stroke

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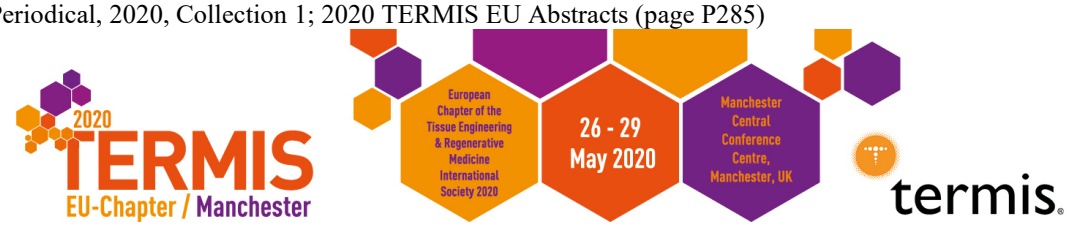
INTRODUCTION:Stroke is a leading cause of death and disability worldwide. Current treatments are focussed on limiting brain damage, while none exist that target brain regeneration to promote functional recovery. After stroke, dead tissue is cleared by immune cells, leaving a fluid-filled cavity, which does not structurally support regeneration, although evidence of neuronal and vascular progenitor cells migrating towards the lesion site exists. The physical support for brain tissue regeneration might be offered by soft, injectable biomaterials which can also deliver therapeutic agents such as cells or growth factors. Self-assembling peptide hydrogels (SAPHs) are of particular interest for brain tissue engineering and regeneration due to their controlled synthesis, biodegradability, non-harmful by-products useful in cell metabolism and tuneable stiffness. For their future use in stroke therapy, peptide monomer immunogenicity and neurotoxicity need to be evaluated, as well as their therapeutic agents release potential.

METHODS:Microglial and endothelial activation, as well as the neurotoxic potential of commercially available SAPHs was examined after incubating brain cells with hydrogel degradation products. Cell viability, lactate dehydrogenase (LDH) activity and secretion of inflammatory molecules such as interleukin-6 (IL-6) were measured. To assess the biocompatibility with brain tissue in vivo, healthy mice were injected in the brain with SAPHs. Microglial activation, apoptosis and phagocytosis were investigated through immunohistochemistry performed on brain sections. Protein release from hydrogels was assessed in vitro following the incubation of SAPHs with therapeutic factors.

RESULTS:Two of the SAPHs candidates, PeptiGel Alpha2 and PuraMatrix, did not significantly affect neuronal, endothelial or microglial cell viability in vitro, did not activate microglial cells and did not induce cell apoptosis in vivo, after intracerebral injection in healthy mice. In contrast, PeptiGel Alpha4 affected neuronal cell viability in vitro and induced microglial activation in vivo. All the SAPHs appeared to induce microglial phagocytosis. Protein release studies revealed that PeptiGel Alpha4 released VEGF (vascular endothelial growth factor) in a sustained manner.

DISCUSSION & CONCLUSIONS:Our data shows that SAPHs are promising biomaterials for their therapeutic use in brain regeneration. Two of the tested hydrogels, PeptiGel Alpha2 and PuraMatrix could be used for cell delivery into the brain and need to be future investigated. PeptiGel Alpha 4 could be explored as a VEGF delivery system.

Keywords: Hydrogels and injectable systems, In vivo and animal models



The chick embryo as a reductionist model to study biomaterial-based therapies targeting spinal cord injury

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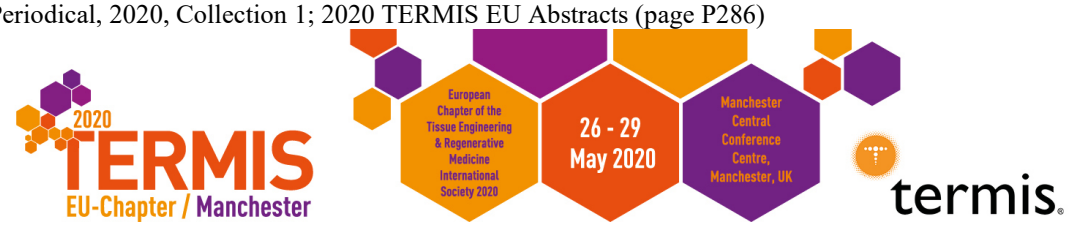
INTRODUCTION:Effective repair of spinal cord injury (SCI) remains a considerable challenge due to multiple regenerative barriers including tissue scarring and an inhibitory molecular environment. Therapeutic biomaterial implantation can potentially address these challenges by stimulating nerve fibre regeneration, reducing tissue scarring and/or acting as carriers for drugs or therapeutic cell populations. Whilst promising, research examining biomaterial implantation into SCI currently relies on the use of complex, adult animal models which are labour and cost intensive and associated with substantial ethical issues.
As a potential alternative, the chick embryo spinal cord possesses the same main cell types and structure as a mammal and it does not regenerate after injuries inflicted later than embryonic day 13 (E13), similarly to an adult. Moreover, the oviparous nature of the chick allows for easy access and manipulation of the embryo while sparing the mother's life and the embryo is not considered a protected animal until E14, reducing ethical concerns. Despite this, use of the chick embryo as a model in which to test new biomaterial-based therapies targeting SCI has not yet been attempted. Here, we aimed to establish an organotypic SCI slice culture model from the chick embryo based on those previously established in rodent tissue. Specifically, we aimed to (i) characterise the pathological responses of the slice to injury and (ii) examine the regenerative capacity of a clinically available collagen biomaterial (HemopatchTM) when implanted in the injury site.

METHODS:We cultured spinal cord organotypic slices from E14 chick embryos and introduced a transecting injury after 4 days. Some slices received implantation of acellular HemopatchTM in the injury gap instantly or 12 days later. Slices were fixed at different time-points post-injury and pathological responses were observed using immunofluorescence.

RESULTS:We were able to produce highly viable slices grown up to 38 days that mimic some of the main features of a mammalian adult SCI, including, poor nerve regeneration, generation of a glial scar, demyelination and microglial infiltration. Further, implantation of a collagen biomaterial resulted in enhanced nerve fibre growth into the lesion site, invasion of astrocytes and a reduction of the glial scar.

DISCUSSION & CONCLUSIONS:These findings suggest that organotypic slices derived from the chick embryo spinal cord could be used as an early stage screening tool to test biomaterial-based treatment of SCI. This has potential impact within the 3Rs by highlighting promising materials early in development before progressing to more complex mammalian models.

Keywords: In vivo and animal models, Biomaterials



Collagen-based cellular microenvironments for brain repair after ischemic stoke

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INTRODUCTION:Disruption of cerebral blood flow in the brain during ischemic stroke results in loss of nervous tissue leading to severe disability or death (1). Currently, the limited treatments available create the need for new therapeutic interventions for brain regeneration. Stem cell transplantation is an emerging avenue aiming to repair the damaged brain (2). Additionally, extracellular matrix (ECM)-based hydrogels are used in combination to boost stem cell survival and integration into the host, which is a limitation of stem-cell based therapies (3). It is hypothesised that a 3D scaffold can support functional integration of neural stem cells and endothelial cells following stroke to re-establish neural networks and promote reconstruction of damaged vasculature respectively. For this, we examine whether combinations of vascular and neural cells in a 3D collagen hydrogel can be used to in vitro model the neurovascular tissue.

METHODS:Collagen I-based hydrogels were formed at 37°C and functionalised by incorporation of ECM proteins (Collagen IV, Laminin, Fibronectin, Vitronectin). Hydrogel mechanical properties were assessed by oscillatory rheology. The ability of the gels to support 3D neurovascular network formation was assessed by encapsulating induced pluripotent stem cell-derived neural progenitor cells (IPSC-NPCs) and the brain endothelial cell line hCMEC/D3 into the gels. The 3D constructs were assessed for cell viability and cytotoxicity, cell morphology and network formation.

RESULTS:Collagen-based hydrogels can be successfully functionalised using ECM proteins and have mechanical properties compatible for application in the brain. The hydrogels are shown to be biocompatible and have low cytotoxicity while supporting the formation of rudimentary neuronal and vascular networks.

DISCUSSION & CONCLUSIONS:Collagen-based hydrogels can be encapsulated with neuronal and vascular cells for the formation of in vitro neurovascular microenvironments. Subsequently, it is of interest to assess the injectable neurovascular constructs for the reestablishment of neurovascular networks in an animal model of ischemic stroke as a potential hydrogel-based cell transplantation therapy for brain regeneration.

ACKNOWLEDGEMENTS:Financial support was provided by the EPSRC & MRC (EP/L014904/1)

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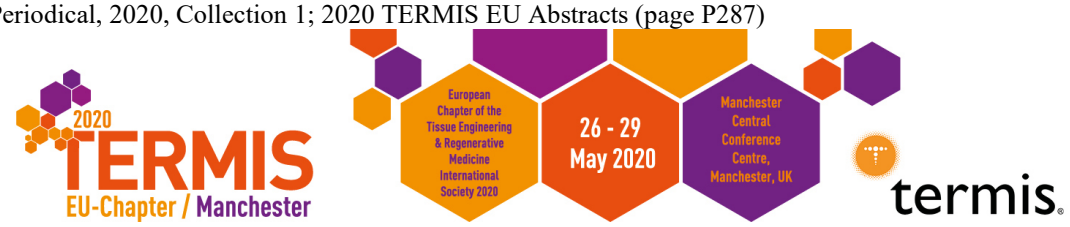
Keywords: In vitro microenvironments, Induced pluripotent stem cells



Reverse Engineering a Human 3D-Blood Brain Barrier Model for Investigation of Vascular Dysfunction in Dementia

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INTRODUCTION:The blood-brain barrier (BBB) is a key interface between the neural and vascular components of the neurovascular unit (NVU). Dysfunction of the BBB precedes and exacerbates many different dementias. To investigate underlying mechanisms of NVU dysfunction there is a need to replicate the 3D architecture of the NVU in vitro; with both biophysical and biochemical interactions between cells types and the extracellular matrix (ECM) being important to tissue function. In this study collagen hydrogel and Matrigel matrices are used as a platform for modelling a functional 3D-BBB, with interactions between a brain microvascular endothelial cell (BMEC) monolayer and a neuron-rich 3D component – with both cell types derived from human induced pluripotent stem cells (iPSCs) – aiming to produce a more clinically relevant 3D-BBB model.

METHODS:Neurons were encapsulated into collagen hydrogels at day 40 of the neuron differentiation protocol and BMECs were seeded on top of the neuron-laden hydrogel at day 8 of the BMEC differentiation protocol (neuron D-60). BBB-BMEC monolayer functionality, with and without neurons encapsulated in the collagen hydrogel, was determined using trans-endothelial electrical resistance (TEER) and sodium fluorescein permeability assays, along with immunostaining for tight junction (TJ) protein expression. ECM based protein coating of tissue culture surfaces was optimised for BBB phenotype using collagen IV and fibronectin (Col-Fn) and Matrigel.

RESULTS:Comparison of BMECs grown on Col-Fn or Matrigel showed no significant difference in permeability, or TJ protein localisation and expression. Co-culture of neurons with BMECs significantly decreased the permeability of the BBB-BMEC monolayer.

DISCUSSION & CONCLUSIONS:The developed human 3D-BBB model allows for biophysical and biochemical interactions between neurons and BMECs, whilst replicating the physical properties of the brain with highly functional BBB phenotype. Further work will utilise the reverse engineering capability of this model to investigate disease mechanisms in dementia.

ACKNOWLEDGEMENTS:MRC and EPSRC for funding the CDT in Regenerative Medicine program. My supervisors: N Hooper, T Wang, and M Domingos; and lab colleagues. Grant reference number: EP/L014904/1

Keywords: Nervous system (brain-central-peripheral / disorders), Disease models

The regenerative potential of injectable peptide hydrogels for intracerebral haemorrhage therapy

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INTRODUCTION:Intracerebral haemorrhage (ICH) is a subtype of stroke and makes up approximately 10-15% of cases. Although ICH is the deadliest form of stroke, current interventions are limited to best medical management and surgical intervention. ICH survivors often suffer life-changing motor and cognitive impairments. The significant unmet clinical need and socioeconomic burden of stroke, means innovative tissue engineering approaches are gaining interest. The acute loss of tissue and poor level of endogenous regeneration makes ICH an attractive target for regenerative medicine. To facilitate regeneration of the stroke cavity, injectable hydrogels are proposed, as they can be designed to mimic properties of healthy neural tissue, act as delivery platforms for therapeutics, and provide a scaffold for regeneration.

METHODS:In a rat model of ICH, self-assembling peptide hydrogels (SAPH) from Manchester BIOGEL® were injected intralesionally, during the chronic phase of the disease (day 7). The aim was to determine the safety and feasibility of SAPH injection in the context of ICH, and to determine whether the material alone would exert effects. To compare functional outcomes of the hydrogel injected group (n=8) with the ICH only (n=7) and sham-injected (n=6) groups, various measures of sensorimotor function were assessed over 28 days, including neurological scoring and cylinder tasks. To determine the effect of SAPH injection on the adjacent injured and healthy brain tissue, immunohistochemical markers were chosen to study cell death and inflammation at the injection site, as well as regenerative markers of neurogenesis and angiogenesis.

RESULTS:Initial results suggest that intracerebral SAPH injection after ICH is well tolerated. Host cells were able to infiltrate the hydrogel, and there was no marked exacerbation of inflammatory response or glial scarring in response to SAPH injection. Functionally, there was no beneficial effect of SAPH injection compared to the ICH only group. In the cylinder task, hydrogel injection appeared to worsen post-stroke asymmetry, yet on average this group had larger initial haemorrhage volumes compared to the ICH only group (37.99µl ± 11.1 vs. 28.92 µl ± 10.17).

DISCUSSION & CONCLUSIONS:The lack of a beneficial effect of hydrogel injection alone, without encapsulation of a therapeutic entity such as stem cells, was expected. The current work demonstrates that SAPH injection does not cause a significant exacerbation of ICH outcomes in rats. The infiltration of host cells into the hydrogel is promising. Future work will use the SAPHs to deliver mesenchymal stem cell secretome, to stimulate repair of the surrounding tissue.

Keywords: Hydrogels and injectable systems, In vivo and animal models

Quantative Evaluation of Regeneration-Related Intrafascicular Components in Decellularized Allograft Nerve Strategies. An in vivo Analysis

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INTRODUCTION:Critical nerve transections are clinically repaired by autograft techniques. However, these techniques, considered the gold standard, have very well-known disadvantages. Tissue engineering strategies try to overcome these limitations. Decellularized nerves can be used to bridge nerve stumps as they provide natural nerve architecture and extracellular matrix components to enhance in vivo regeneration. The aim of this study is to quantify the main intrafascicular components playing a role in peripheral nerve regeneration process using three different chemically decellularized nerve allografts.

METHODS:18 Wistar rats were anaesthetized and a 10-mm sciatic nerve defect was created and repaired by using previously described decellularized nerves: Sondel (SD) (1), Hudson (HD) and Roosens (RSN) methods (n=6 each). After 12 weeks, rats were perfused and the implanted grafts were harvested, fixed, embedded in paraffin and sections were stained with MCOLL histochemical method. Afterwards, myelin and collagen expressions were measured and quantified using ImageJ software.

RESULTS:The quantitative evaluation of intrafascicular collagen fibers and myelin sheets showed differences among the experimental groups. HD group showed the lowest collagen expression and the highest myelin formation 12 weeks post-trauma, being these differences statistically significant with SD group, which showed the lowest myelin formation and the highest collagen expression. The endoneural components in RSN group did not reach HD group results, although differences were not statistically significant.

DISCUSSION & CONCLUSIONS:Analysis of the endoneural changes in peripheral nerve repair is a useful tool to determine nerve regeneration. On the one hand, collagen expression is related to a fibrotic process that could, in a final term, obstruct axons sprouting into appropriate distal fascicles that could thereby delay and limit nerve regeneration. On the other hand, myelin formation is directly related to axonal differentiation and maturation, which is an evident consequence of nerve regeneration. Our data indicated that not all decellularized nerve allografts promote myelin sheet formation and in fact, some of them could trigger a fibrotic process that could decrease axon regeneration.

ACKNOWLEDGEMENTS:This study was supported by the Spanish “Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica,” from the National Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), Grant FIS PI17/0393, co-financed by “Fondo Europeo de Desarrollo Regional (FEDER),” European Union.

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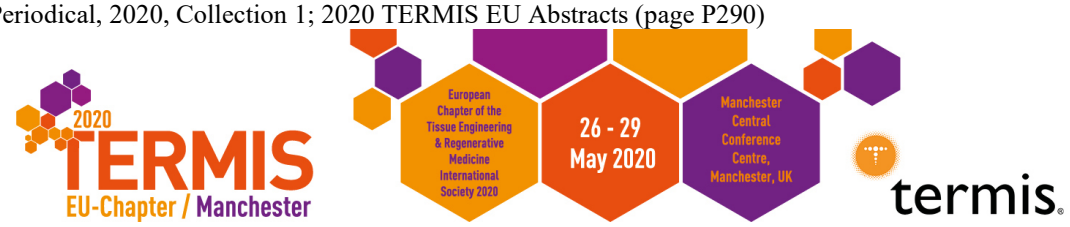
Keywords: Nervous system (brain-central-peripheral / disorders), Biofabrication



Biomechanical and Biological Effects of Genipin Chemical Crosslinking on Decellularized Peripheral Nerves

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INTRODUCTION:Generation of crosslinked-decellularized extracellular matrices emerged as a promising alternative in tissue engineering. These biomaterials may provide a natural, native, well-organized, non-immunogenic, mechanically stable scaffolds for a wide range of tissue engineering applications. However, some chemical crosslinkers can affect the regenerative microenvironment, cell viability and functionality due to cytotoxicity. In this regard, the current study aimed to investigate the ex vivo effects of Genipin (GP) crosslinker on the biomechanical and biological properties of decellularized nerves.

METHODS:Rat sciatic nerves were decellularized with the chemical-enzymatic method of Roosens (1). Then, decellularized nerves were subjected to GP crosslinking at 0.1 and 0.25% as described previously (2). Non-crosslinked decellularized nerves and native healthy nerves were used as control groups (DC-CTR and CTR respectively). Tensile test was performed with an Instron biomechanical analyzer. After several washes, 2x104 rat-Adipose MSCs (ADMSCs) were seeded on the nerves surface. Cell viability and irreversible cell-membrane damage were determined 48h after culture by using Live/Dead Assay and quantification of released DNA, respectively, as described previously (2). In both cases, technical controls were used.

RESULTS:Tensile tests showed a significant increase of stress at fracture values with both concentrations of GP (0.1 and 0.25%) as compared with controls ($p<0.05$). Live/Dead images revealed viable ADMSCs attached to the nerve surface in all experimental groups. Nevertheless, viable cells were more abundant in DC-CTR group than crosslinked nerves. Curiously, quantification of DNA released showed a significant decrease ($p=0.021$) of DNA in medium in 0.1% GP group as compared to DC-CTR, but no differences between both GP groups were found.

DISCUSSION & CONCLUSIONS:This ex vivo study demonstrate that GP enhanced the biomechanical properties of decellularized nerves without a considerable detriment of their biological properties. Indeed, crosslinked decellularized nerves supported cell viability, functionality and attachment ex vivo. However, future preclinical in vivo studies are required to demonstrate the suitability of these crosslinked nerve grafts in peripheral nerve repair.

ACKNOWLEDGEMENTS:This study was Supported by Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) from the Spanish Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grant FIS PI17/0393 (co-financed by ERDF-FEDER, EU).

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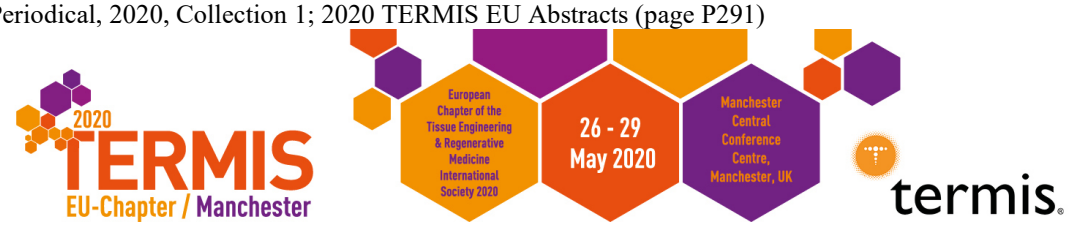
Keywords: Nervous system (brain-central-peripheral / disorders), Decellularised matrices



Novel Biofabrication Technique for the Generation of Nanostructured Neuroconductive Guides Containing Cellular Neuroinductive Hydrogels

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INTRODUCTION:Clinical management of critically damaged peripheral nerves remains challenging. Several tissue engineering strategies has been developed and tested during the last years with promising experimental results. These studies suggest that biomimetic, biologically active nerve substitutes show the best results, which are closely comparable to the gold standard repair technique, the autograft technique. However, more efficient nerve substitutes able to reproduce the fine architecture of the human peripheral nerve, consisting of an internal neuroglial component and an external epineural layer, are needed for these patients (1). The aim of this study was to generate new bioartificial nerve substitutes resembling the complex structure of most human nerves that could efficiently guide and induce nerve tissue regeneration.

METHODS:A composite nerve substitute with an external neuroconductive component and an internal neuroinductive material was generated. For the neuroconductive component, fibrin-agarose hydrogels (FAH) were generated, nanostructured (NFAH), rolled around glass molds and then subjected to genipin crosslinking (NFAH-GP) as previously described (2). Then, FAH containing adipose-derived mesenchymal cells (ADMSCs) were generated, nanostructured and rolled for the generation of compacted and biologically active neuroinductive rods (3). Both components were combined to generate a novel nerve substitute for peripheral nerve repair that was characterized at the biological, structural and biomechanical levels.

RESULTS:Analysis of the novel complex nerves showed a well-structured external layer resembling the epineural layer of the human nerve with internal structures analogue to the internal neuroglial component of the native nerve. From a biomechanical point of view, the NFAH, and specially the NFAH-GP, were suitable for the generation of the neuroconductive guide in a controlled manner. However, NFAH were more biocompatible. The neuroinductive component resulted to be mechanically and structurally stable, and supported ADMSCs viability and functionality ex vivo.

DISCUSSION & CONCLUSIONS:These results suggest that NFAH and specially, NFAH-GP are suitable biomaterials to generate biomechanically stable and biocompatible composite nerve substitutes with potential combined neuroconductive and neuroconductive functions. in vivo preclinical studies are needed to determine their usefulness in peripheral nerve repair.

ACKNOWLEDGEMENTS:This study was supported by the Spanish “Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica,” from the National Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), Grant FIS PI17/0393, co-financed by “Fondo Europeo de Desarrollo Regional (FEDER),” European Union.

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Keywords: Nervous system (brain-central-peripheral / disorders), Biofabrication

In vitro evaluation of extracellular matrix components in two different peripheral nerve decellularization protocols

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INTRODUCTION:Peripheral nerve injuries may require autograft transplantation. This method has disadvantages as an additional injury, loss of sensitivity and donor site morbidity. The decellularization technique have evolved as a solution to overcome the adverse immune reaction provoked by the resident cells in the transplanted allografts meanwhile providing a natural extracellular matrix (ECM) (Han et al., 2019). In this study, we compared the effect of detergents used in previously published decellularization protocols on the natural extracellular matrix components and cell cytotoxicity (Boriani et al., 2017; Sondell, Lundborg, & Kanje, 1998)

METHODS:Rat adult Sciatic nerves were withdrawn and cut into fragments each of 10mm, decellularization protocols were followed. For Sondell protocol, nerve pieces were immersed in distilled water for 7 hours then treated with 3% triton X-100 followed by 4% sodium deoxycholate. Rizzoli protocol nerves were immersed in solution containing125 mM SB-10, 0.2% Triton X-100 and Pen Strep 1% for 48 hours then moved to another solution containing 0.25% SDS and later 6 alternating sonification cycles of 40 Hz ultrasound frequency for 5 min were applied. Nerves from both protocols were washed in PBS and stored in 4ºC till further processing. For general morphology evaluation Haematoxylin-Eosin (HE), S100 and vimentin were performed. Picrosirius red (Picro) and alcian blue (AB) staining were performed to detect the ECM components collagen and proteoglycans respectively.

RESULTS:Rizzoli has a better general structure than Sondell but it showed S100 and vimentin signal in the middle part of the decellularized nerves, that was absent in the nerve's outer part and completely absent from Sondell. Both showed myelin remnants by Mcoll staining, no great differences were shown in picro and AB staining. Cells showed better viability on Sondell nerve substrates.

DISCUSSION & CONCLUSIONS:Rizzoli conserved a better overall nerve structure, but the decellularization method was not sufficient to penetrate to the central part of the nerve. Sondell showed a destructed structure but had a better cell viability when seeded. Modifications to the protocols could be done to obtain a good penetration and cell removal while preserving the general nerve structure.

ACKNOWLEDGEMENTS:This study was Supported by Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) from the Spanish Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grant FIS PI17/0393 (co-financed by ERDF-FEDER, EU).

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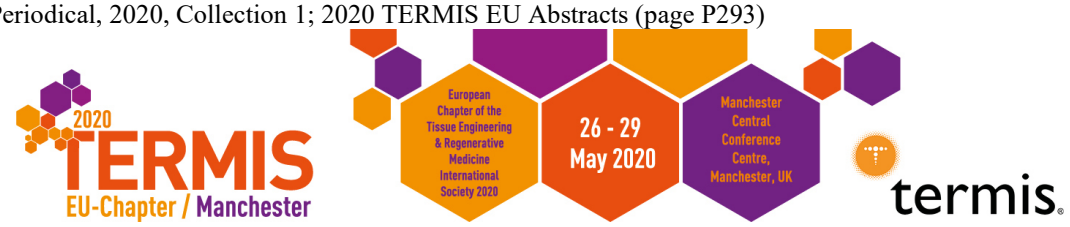
Keywords: Nervous system (brain-central-peripheral / disorders), Decellularised matrices



Mapping the amnion proteome – a step towards designing healing-triggering biomaterials to prevent iPPROM

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INTRODUCTION:Preterm birth is the main cause of mortality worldwide for children under 5 years old, with an estimated 1 million newborns dying every year because of direct complications associated with preterm birth [1]. One of the causes of preterm birth is the iatrogenic premature preterm rupture of the fetal membranes (iPPROM), which may occur after a minimally invasive intervention in the amniotic cavity. Previous studies showed that the fetal membranes (FM) do not possess the ability to spontaneously heal the defect created during fetoscopy [2], possibly due to their poor vascularization [3]. Restoring their integrity after intervention could help in the development of a healthy fetus and newborn and would be a stepping-stone towards solving an unmet clinical need. In order to design tailored healing-triggering biomaterials, an exhaustive investigation of the composition of the FM, which have been poorly characterized in the past [4-6] is needed.

METHODS:We first optimized the method for amniotic protein extraction by comparing two protein extraction, FASP and in solution. We then made use of a multidimensional proteomics strategy by combining a high pH reversed-phase liquid chromatography (RPLC) followed by a low pH LC-MS/MS analysis in order to have deep proteome profiling of the human amnion.

RESULTS:More than 300 proteins were better extracted with the FASP method. Further fractionation of the sample prior to LC-MS/MS enabled the identification of over 5000 proteins in the amniotic membrane. An overrepresentation analysis depicted extracellular matrix (ECM) in the top 10 enriched categories, with 298 ECM proteins identified. Quantification of protein normalized abundance showed specific core matrisome and matrisome-associated proteins among the top 2% most abundant proteins in the human amnion.

DISCUSSION & CONCLUSIONS:To our knowledge, this is the most complete amnion proteomic characterization existing so far and the first attempt to broadly quantify its composition. We consider that the knowledge derived from it could be of great help in the development of biomaterials for the prevention of iPPROM. Further studies will include the confirmation of the findings by histology and the analysis of mid-term pregnancy samples.

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Keywords: Wound healing, Other

Ex vivo model of cartilage repair for testing regenerative potential of chondrospheres

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INTRODUCTION:Tissue spheroids are densely packed cell aggregates which have been proposed to use as building blocks in tissue engineering [1]. Moreover, certain types of tissue spheroids such as chondrospheres are already used in clinical practice for treatment cartilage defects [2]. Ex vivo assay for testing regenerative potential of chondrospheres for cartilage repair have been recently developed [3]. Here we report about development of first time a quantitative and predictive ex vivo assay of cartilage repair for testing potential molecules and compositions which could enhance regenerative potential of chondrospheres.

METHODS:Primary culture of ovine chondrocytes have been used. Chondrospheres of standard size and shape have been produced using non-adhesive multiwells (Corning, USA). Viability of produced chondrospheres have been estimated using LIVE/DEAD Assay. For estimation of regenerative potential of chondrospheres they have been placed into small defect created in isolated fragment of ovine cartilage. The kinetics of tissue spheroid spreading on the surface of injured cartilage and kinetics of adjacent chondrospheres fusion have been estimated. Histology, immunohistochemistry and scanning electron microscopy have been used for morphological analysis of cartilage regeneration ex vivo. The kinetics of chondrospheres spreading have been estimated on histological sections during 7 days. The percent of cartilage surface covered by attached and spread chondrospheres have been used as quantitative criteria of their regenerative potential ex vivo.

RESULTS:It have been demonstrated that chondrospheres attached and spread on the surface of injured cartilage and fused with each other and formed continuous tissue engineered construct integrated with cartilage fragment. It have been shown that densely placed chondrospheres completely regenerate the cartilage defect. Combined estimation of chondrospheres spreading kinetics together with estimation the kinetics of their tissue fusion by measuring of fusion area allows to perform quantitative estimation of their regenerative potential ex vivo and predict chondrospheres regenerative potential in vivo.

DISCUSSION & CONCLUSIONS:A novel quantitative ex vivo assay of cartilage repair for testing regenerative potential of chondrospheres have been developed. It allows to predict regenerative potential of chondrospheres and could be used for systematic screening of potential molecules which could enhance regenerative potential of chondrospheres.

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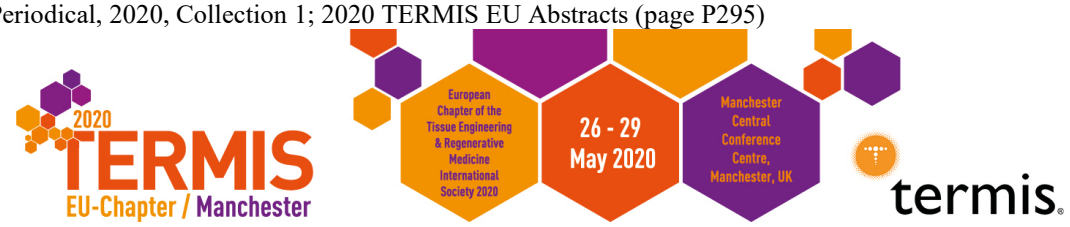
Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Cartilage / joint and arthritic conditions



Hydrogel Development for On-Chip Modelling of Human Bone

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INTRODUCTION:Development of therapeutics for bone disease currently rely on simple 2D in vitro assays, and subsequent complex in vivo models. In vitro culture can be too far removed from the in vivo condition, and while animal models are substantially more complex, differences between species often questions their validity (1). To bridge the complexity between these two models, microphysiological systems (MPS) have been developed to mimic key features of human organs at the microscale. The objective of this study was to develop a hydrogel system that facilitates human bone tissue development within a MPS, recreating the key cellular and extracellular markers of human bone.

METHODS:Screening:
Bone Marrow Stromal Cells (MSCs) (Lonza, USA) were cultured in rat tail type 1 collagen gels (Corning, Germany) for two weeks. Design of experiments (DOE) methods were used to screen the effects of 6 culture parameters on osteocytogenic differentiation; collagen matrix stiffness, osteogenic supplementation; nano-hydroxyapatite (nHA) concentration, oxygen tension, cell density, and retinoic acid concentration. Differentiation was evaluated by cell morphology, ALP expression, and secreted Sclerostin levels at day 14.

Optimisation:
For optimisation experiments, matrix stiffness and nHA content were further evaluated. To enhance matrix stiffness, collagen gels were reinforced with Alginate (Pronova, Norway) to produce stiffer interpenetrating network (IPN) gels. MSC culture in the IPNs was evaluated for osteocytogenesis via cell morphology, expression of osteocyte markers PDPN and DMP-1, in addition to mineral deposition.

RESULTS:The DOE revealed collagen matrix stiffness and nHA concentration had the largest effect on osteocytogenesis of MSCs after 2 weeks of culture. In the proceeding optimisation experiment, MSCs in the least stiff collagen only gels produced a highly mineralised matrix, significantly higher amounts of sclerostin, and were positive for osteocyte markers E11 and DMP-1. Incorporation of alginate seemed to abrogate sclerostin production, but facilitated the development of a highly dendritic osteocyte-like morphology.

DISCUSSION & CONCLUSIONS:We found that DOE methods could be used to identify and iteratively develop culture conditions that promote differentiation of MSCs along an osteogenic pathway, with MSCs encapsulated within soft collagen-nHa gels exhibiting an osteocyte-like signature. Complex 3D models of human bone models are currently lacking and this report describes a method of generating the key features of human bone for incorporation in a MPS. Such organ-on-chip platforms will transform research into the (patho)physiology of bone and accelerate the development of new treatments for damaged and diseased tissue.

Acknowledgements:SFI Grant Number 13/RC/2073

References:Ledford et al., Nature News 447:526-528, 2011

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, Bone and bone disorders (osteoporosis etc)

Combining organs-on-chips, biofabrication and software tools: the path to automation and complexity

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INTRODUCTION:Biofabrication is increasingly seen as the ideal solution for generating in vitro models. Not only it allows to accurately deposit and assemble specific materials (including cells) into complex 3D constructs but also allows to culture, stimulate, and analyze them into highly controlled and dynamic environments. Given the complexity of the human body, it becomes necessary to develop tools which are able to automatically generate body-mimicking architectures and environments, particularly in a body-on-chip fashion. The purpose of this work was to demonstrate the limitless ability of newly developed software tools to generate body-like structures and environments and in this way democratize the access to organ-on-chip technologies.

METHODS:Parametric design methodologies were employed in order to enable automated generation of 3D device designs upon insertion of user-defined settings. Resulting parametrically designed shapes were then physically materialized by employing additive manufacturing and bioreactor know-how in order to produce fully functioning organ-on-chip devices which may be composed of an array of customizable designs and features.

RESULTS:It was possible to demonstrate that parametric design methodologies combined with additive manufacturing and bioreactor technologies may allow to easily, intuitively and automatically generate organ-on-chip devices with endless degrees of complexity, which may be employed in a wide array of applications.

DISCUSSION & CONCLUSIONS:The combination of “smart” software tools with highly automated technologies such as additive manufacturing and bioreactors may lead the way to democratizing the access to organ-on-chip technologies. Furthermore, it may as well open new avenues towards a deeper understanding of the human body by means of a greater ability to mimic its physiology and morphology under in vitro controlled environments.
Acknowledgements:This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 798014.

Keywords: 3D printing and bioprinting, Biofabrication

Biomaterial-based functional microenvironments as models for in vitro testing

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INTRODUCTION:11 million animals are used every year in the EU for research, education and testing. Many results using animal models do not actually translate to better human healthcare or an understanding of the human body. Scientists also struggle with the ethics of using animals, but our attempts at finding alternatives are often thwarted by aspects such as funding and the publishing process. We need a change of paradigm. Our work in biomaterials - while aiming chiefly at improving human health - has the added bonus of providing efficient alternatives for some of these animal models, contributing ultimately to a full 'human-on-a-chip'. Here we present some of the biomaterials that we are engineering: they mimic infarcted cardiac tissue, the hippocampus, and even recreate the process of angiogenesis 'on-a-chip', as well as leading to a better understanding of how cells exert forces.

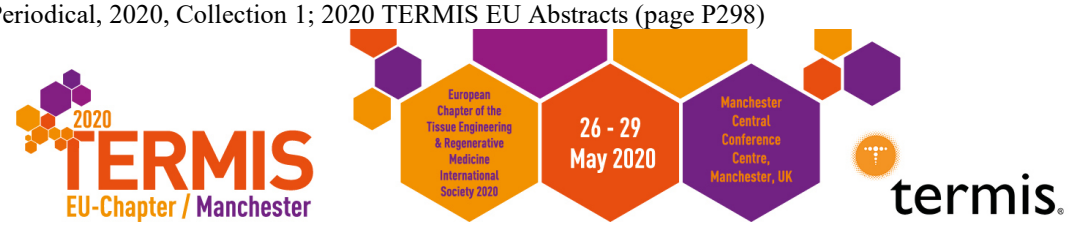
METHODS:Several microenvironments were fabricated based on biocompatible hydrogels, such as fibrin, and/or electrospun surface modified polymeric fibers, such as polylactic acid. Microfluidics was also used to support the size and conditions of the microenvironments. Finite elements simulation was used to narrow down experimental conditions and validate feasibility of the microfluidic layouts and gradients. In the case of the angiogenesis-on-a-chip model, in silico simulations were also performed to shed some light on the processes that govern the formation of new blood vessels.

RESULTS:Experimental microenvironments were fully validated using fluorochromophores, latex beads for flow rates and external probes to monitor the different applications. The constructs showed that the biomaterials can mimic natural 3D biological environments. They also demonstrated their versatility to be adapted to individual patients, clinicians' differing requirements and the isolation and/or elimination of variables for strictly parameter-controlled microenvironments.

DISCUSSION & CONCLUSIONS:The gap of knowledge between in situ and in vitro testing is becoming narrower every day. The development of new markers and microscopes makes it easier to obtain basic biomolecular information. There´s still a long way to go, but these models complement much of the information given by conventional in situ and in vivo tests. They are questioning and helping to refine many of the theories established in the biomaterials field so far. Animal experiments should be minimized and controlled microenvironment are a serious candidate to replace most of them.

Acknowledgements:We thank the MICIU for their funding: RTI2018-097038-B-C21 and -C22, RTI2018-096320-B-C21 and FPU17/06161.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, In vitro microenvironments



Autonomous spheroid formation from monolayer by growth surface compartmentation

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INTRODUCTION:Scaffold free spheroid cultures are the standard culture model for in vitro application, but are also gaining importance in clinics. Shortcomings of current spheroid generation systems are high time/reagent consumption and the lack of early readout parameters. Here we introduce an efficient system for generating chondrogenic spheroids via self-assembling from cell monolayer.

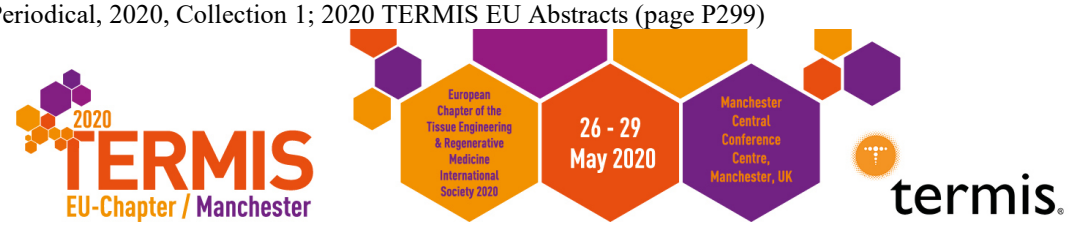
METHODS:Cell culture dishes were compartmentalized in different grid sizes using CO_2 laser. Telomerase immortalized adipose derived stromal/stem cells (ASC/TERT1) alone and in co-culture with primary human articular chondrocytes (hAC) were seeded at different ratios. Cells were cultivated in low dose differentiation media containing 1ng/ml BMP-6 and TGFβ-3. Spheroid size control via grid size variation, formation speed and cell type distribution using live cell labelling (DiO, mCherry) were assessed using time-laps imaging and light-/confocal microscopy. Spheroid characteristics were compared to standard pellet culture using qRT-PCR (collagen type 1/2 (Col1/2)) and (immuno)histology (Alcian blue, Col2). Spheroids were embedded into fibrin and cellular outgrowth/matrix deposition were assessed to model tissue regeneration.

RESULTS:Grid size variation allowed for control of spheroid size (mean diameter for grid size 3mm = 340.9µm and for 1mm = 134.4µm). In ASC/TERT1 monoculture spheroid was completed after 3 weeks and shortened to 1-2 weeks in co-cultures with hAC, promoting significantly faster formation with increasing ratio ($p<0.05$ and 0.01 for 1:1 and 1:4 ASC/TERT1:hAC ratio respectively). Cellular distribution analysis using hAC and ASC/TERT1 labelled with DiO and mCherry respectively, showed spheroid cortices predominantly formed by aligned ASC/TERT1 and spheroid interiors populated by a mixed populations. (Immuno-)histology of grid spheroids and pellet culture displayed differences in matrix distribution. Grid spheroids, especially in ASC/TERT1 mono-cultures, showed sheet-like matrix depositions and thin cortex regions while pellet culture exhibited more dispersed matrix and thicker cortex regions. qRT PCR and (immuno-)histology showed similar amounts of Col1/2 expression and Col2 and GAG deposition in both systems. When embedding spheroids into fibrin, cellular outgrowth lead to matrix deposition into the hydrogel, especially co-culture.

DISCUSSION & CONCLUSIONS:The data herein presented show an efficient system for cell spheroid self-assembly from monolayer with controllable spheroid size and similar chondrogenic potential as standard pellet culture, while reducing time/media consumption. The spheroid inducer system therefore has a high potential for cartilage regeneration applications in vitro as well as in clinics.

Acknowledgements:Funding was received from FFG grant #867720. ASC/TERT1 were kindly provided by Evercyte GmbH.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models, In vitro microenvironments



Silk fibroin-based 3D dynamic breast cancer model to investigate the role of mesenchymal stem cells in the tumor progression

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INTRODUCTION:Tumor progression is driven by the interaction among cells and microenvironment [1]. Recently, mesenchymal stem cells (MSCs) gain attention due to the dual role that they play in cancer. From one side, they promote angiogenesis and impair immune surveillance [2-3]. On the other side, MSCs could prevent cancer invasion [3]. Dynamic in vitro cancer models are needed to explore the mechanisms of cancer progression [4]. In this study, we aim to investigate the secretome modulation of MSCs in breast cancer using a dynamic millifluidic system.

METHODS:Silk fibroin (SF) 2% freeze-dried scaffold with the diameter of 15 mm are prepared. Two chambers of the LIVEBOX 1 reactor (IVTech, Italy) are serially connected to a peristaltic pump. In each chamber, the SF scaffold is seeded with MSCs cells and the coculture of 3D-HMF/MDA-MB-231. Single cultures (3D-MSCs and 3D-HMF/MDA-MB-231) are considered as control. The cell cultures are carried out for 6 days and the flow-rate for the LIVEBOX 1 is set at 50 µl/min. The cell viability is assayed by LIVE/DEAD assay. The cell morphology is observed by means of DAPI/Phalloidin staining. Cytokines and cancer related protein arrays are carried out on the supernatant of the dynamic culture, both in single and co-culture conditions. The results obtained by the protein arrays are validated by mean of RT-PCR.

RESULTS:Herein, we have demonstrated that dynamic condition allows a better cell viability along the 7 days of culture, with cells occupying the whole scaffold. It has been found a modulation in the cytokines profile when 3D-MSCs are in a paracrine contact with 3D-HMF/MDA-MB-231. The protein arrays have shown the upregulations of IL-6, IL-8, MMP-2, MMP-3, Tenascin-C and VEGF in the secretome of 3D-MSCs when they have been co-cultured with the tumor microenvironment, represented by the 3D-HMF/MDA-MB-231 coculture.

DISCUSSION & CONCLUSIONS:The presence of the cancer cells affects the expression of cancer-related markers and the inflammatory proteins in the 3D-MSCs. The 3D in vitro model developed in this work is a step forward for the use of realistic disease model and to understand cancer progression and metastasis.

ACKNOWLEDGEMENTS:This work is supported by EU-2020 grant FoReCaST (H2020-WIDESPREAD-2014-668983) and by BREAST-IT FCT project (PTDC/BTM-ORG/28168/2017)

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Keywords: In vitro microenvironments, Cancer

Multiphoton laser lithography to promote controlled microscale vascular network creation

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INTRODUCTION:Controlled angiogenesis in organ-on-a-chip devices is necessary to both precisely model and control nutrients, hormones, and growth factors transport into the model tissue. Up to now, the only approaches demonstrated rely either on low resolution techniques for channels creation [1,2], or on spontaneous uncontrolled angiogenesis [3].
In order to fill this gap, we exploit multiphoton lithography techniques, to produce structures directly within microfluidic chips with resolution at multiple length scales.

METHODS:Multiphoton lithography is performed through a custom setup based on an infrared femtosecond laser and a fast galvo-scanner. The laser, focused through a microscope objective, has sufficient energy density for multiphoton processes only in the objective focal spot.
A photo-labile O-nb based linker is used in a thiolated gelatin [4]. Femtosecond laser irradiation can selectively cleave the linker, and a successive washing with PBS can empty the irradiated volume.
Molecules with azide photoreactive groups are grafted to the polymer backbone of a methacrylated gelatin [5], locally modifying its stiffness and hydrophilicity.
Human umbilical cord endothelial cells have been incorporated in the methacrylated hydrogel matrix, cultured with cell medium containing vascular endothelial growth factors.

RESULTS:We demonstrate successful cleaving of branched channels in chip, with a feature size from 10 to 100 µm, and a micrometric resolution, using a photosensitizer to reduce the cleaving power threshold.
We created well confined spatial regions modified through multiphoton grafting in hydrogel loaded with encapsulated endothelial cells, and observed their orientation along the microstructured features.

DISCUSSION & CONCLUSIONS:With our results, we propose a set of multiphoton lithography techniques to guide endothelial cell invasion of a hydrogel along predetermined paths, and to promote and support the creation of a vascular network, suitable for fabrications inside a microfluidic chip.

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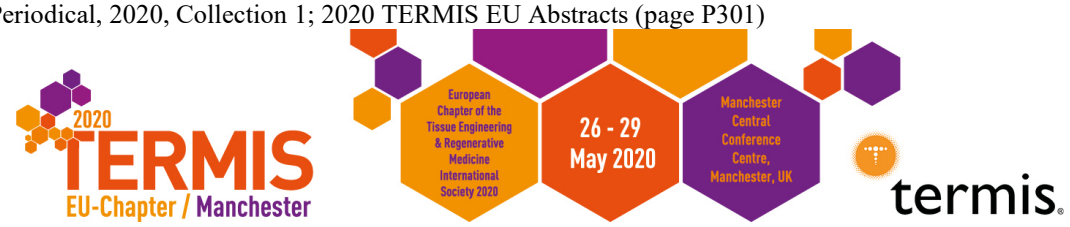
Keywords: 3D printing and bioprinting, Biomaterials



Modeling hyperglycemia using a vessel-on-a-chip technology and endothelial progenitor cells

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INTRODUCTION:Studying hyperglycemia-induced vascular dysfunction and methods to restore the endothelium are important aims in diabetes. This is better replicated in a 3D-format that mimics the physiological structure of the vessel and incorporates cell-cell and cell-matrix interaction. Organ-on-a-chip(OOAC) allows drug testing on organ micro-models developed using human cells under microphysiological conditions, thus marinating human genetic background under complex physiological settings. A potential cell source to screen drugs using OOAC is blood endothelial progenitor cells(EPCs), which are easy to isolate with high proliferative capacity, providing an autologous source for personalized drug testing. The aim of the current work is to (i)develop 3D vessels using human blood outgrowth endothelial cells (BOECs) expanded form EPCs and study hyperglycemia effects on vessel formation and function in an OOAC model, (ii)test the effect of the antidiabetic drug "metformin" and the endothelial nitric oxide synthase (eNOS)-restoring drug "sildenafil" on vessel proliferation and function. METHODS:BOECs were isolated form healthy donors using selective plating1. To develop 3D vessels, an OrganoPlate® 2-lane system(MIMTAS) was used. Neutralised Type I collagen(4mg/ml) was seeded into the gel channel and was polymerized for 15 min, followed by seeding of pre-stained BOECs (20,000cells/well) into the perfusion channel. Plates were left standing at 75°angle to allow cell adhesion to the gel layer, and then incubated on a rocker to allow media perfusion. Vessels were cultured in normal(5.5mM) or high glucose(25mM) EGM-2 media for 72hr in the presence or absence of metformin(50uM) or sildenafil(10uM). Vessel formation was examined by confocal imaging using z-stacks, and viability was assessed using alamarBlue proliferation assay. Barrier-function was assessed by tracking the leakage of fluorescently-tagged-dextrans from the vessel to the gel channel.

RESULTS:BOECs were able to form 3D vessels under both normoglycemic and hyperglycemic conditions, with slower formation under hyperglycemic conditions. This was also reflected in vessels' viability which was reduced under hyperglycemic conditions at 72hr. Treatments with metformin and sildenafil for 72hr increased cell proliferation in both conditions(n=4). Our preliminary data showed an increased permeability to 20KDa FITC-dextran in vessels chronically exposed to hyperglycemia, with sildenafil reducing permeability under these conditions.

DISCUSSION & CONCLUSIONS:Herein we have modeled hyperglycemia using 3D vessel-on-a-chip made of EPCs. The responses of the vessel to hyperglycemia in terms of proliferation and permeability reflected the current knowledge about vascular endothelium. Further investigations are ongoing to validate the model and to test the effects of hyperglycemia/drug treatments on cytokine release, cell adhesion molecules, endothelial markers and oxidative stress.
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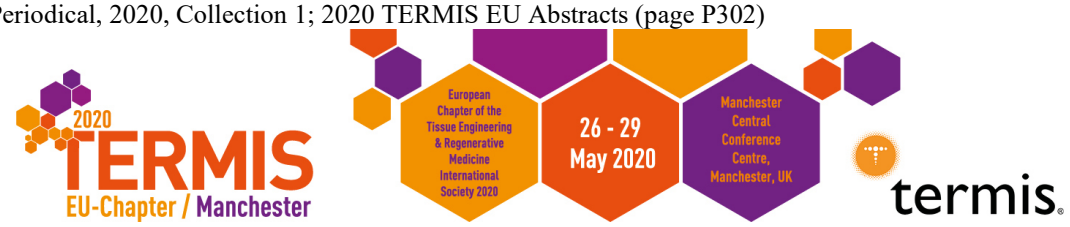
Keywords: Disease models



Bioprinting of iPSC-derived retinal ganglion cells and cortical neurons for the studying of the progression of the neurodegeneration in Alzheimer's disease

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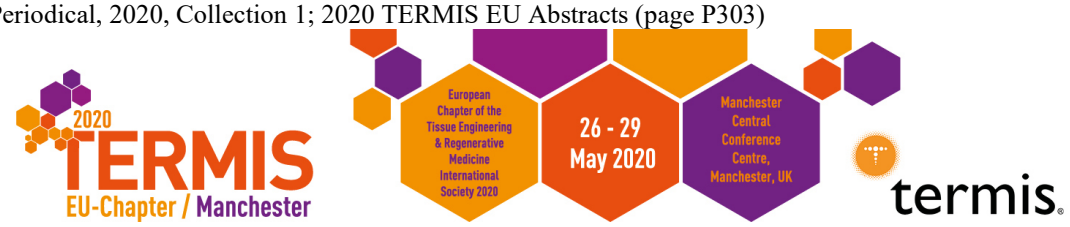
INTRODUCTION:Alzheimer's disease (AD) is the primary cause of dementia in the elderly. The accumulation in either intracellular or extracellular environment of protein-rich aggregates plays a pivotal role in the progression of AD. Although most factors involved in the disease are well known, a clear idea of the biological mechanisms at the basis of the AD onset is still missing. Previous work has reported the spreading of mutated misfolded Tau proteins between neurons in a prion-like manner through the anatomically connected brain regions. Recently, studies carried out on postmortem human retina samples and on rodent retina have highlighted the identification of markers for AD in the retina tissue confirming the spreading of tau oligomers in neighbor areas. However, our knowledge of the neuropathological changes that occur in vivo at pre-symptomatic stages in humans is still uncomplete.

METHODS:The establishment of efficient protocols for the generation of induced pluripotent stem cells (iPSC) starting from patient-isolated somatic cells has given the opportunity to study in in-vitro 2D cell cultures the pathogenesis of a great variety of human brain disorders including AD, Parkinson's disease, and autism-linked disorders. A human iPSC control line and its AD-related isogenic iPSC line are differentiated in vitro to generate both retinal ganglion cells and cortical neurons and then are used for modeling the connection between brain and retina through an extrusion-based printing process which allows the cell and ECM deposition without impairing cell viability in the short and long term.

RESULTS:Herein, the aim of our research work is to develop a hierarchical 3D in vitro model culturing retinal ganglion and cortical neurons to investigate the tau oligomers diffusion in vitro into a much more complex system than the 2D conventional cell culture. This new 3D cellular model may also represent a novel tool for the reliable and safe validation of tailor-made molecules that might be used in the near future for the non-invasive investigation and detection of the retinal neurodegeneration features associated with AD and tauopathies.

DISCUSSION & CONCLUSIONS:We strongly believe that the development of new 3D cellular models based on the use of human stem cells might be taken into consideration for better mimicking the human tissue physiology and the disease onset and progression in vitro, giving an alternative to the use of humanized rodent models and paving the road for non-invasive rapid detection of AD biomarkers.

Keywords: 3D printing and bioprinting, Induced pluripotent stem cells



Dynamic microfluidic platform for generation of cornea organoids

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INTRODUCTION:Corneal tissue engineering aims to fabricate graft equivalents that can restore the experienced visual impairments[1]. Hydrogel based biomaterials have been used to produce corneal tissue equivalents due to their optical and mechanical properties, which can mimic native cornea in vitro[2]. Emerged stem cell technologies offer numerous tissue specific cell types from diverse sources including induced pluripotent stem cells. Mechanical forces have been shown to direct the fate and functionality of stem cells[3]. Microfluidic systems provide controlled dynamic environments for in vitro organ on-a-chip tissue equivalents[4]. In this study, we have developed dynamic platform, which enables stem cells to be cultured and differentiated under physiological pressure of the native cornea.

METHODS:Induced pluripotent stem cells (IPSC) have been seeded on decellularized cornea and placed on perfusable hydrogel inside of microfluidic chip. Hydrogels were synthesized using different concentration of polyethyleneglycol (PEG), polyvinyl alcohol (PVA) and alginate to be used as carrier of decellularized cornea (DC) and provide pressure control. IPSCs were differentiated for 20 days until reaching retinal maturation and seeded on DC. Microfluidic device has been fabricated by assembling Poly(methyl methacrylate) sheets and perfused with syringe pump. Generated construct was integrated into microfluidic system and exposed to continuous fluid flow for 5 weeks.

RESULTS:PVA-Gelatin based hydrogels showed significantly higher mechanical stability and perfusion capacity than PEG and alginate-based hydrogels. Decellualrization rate of cornea matrices have been found to be above 85%. Recellularization studies showed homogenous and sustainable cell distribution and differentiation. Long term maintenance and differentiation (up to 5 weeks) of the generated constructs have been successfully achieved. Immunohistological and transmission electron microscopy analyses demonstrate the cornea specific differentiation and collagen fiber orientation.

DISCUSSION & CONCLUSIONS:The developed hydrogel carrier provides suitable perfusion for the cell culture. Designed microfluidic platform provide maturation and differentiation of organoids under dynamic pressure allowing mimicking the native microenvironment of the corneal structures.

ACKNOWLEDGEMENTS:This work is supported by TUBITAK 217S654, TUBA-GEBIP and BAGEP Awards.

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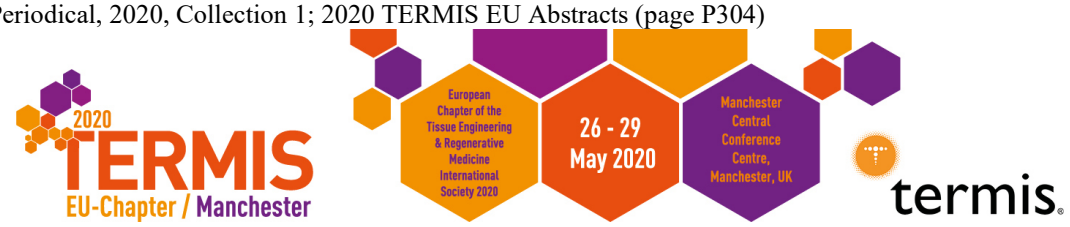
Keywords: Biomaterials, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models



Towards the development of the biohybrid lung: Extracellular matrix synthesis of the membrane-seeded endothelial cells under static and dynamic culture conditions

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INTRODUCTION:To provide an alternative to lung transplantation we aim for the development of a biohybrid lung (BHL), based on contemporary ECMO technology using gas exchange hollow fiber membranes (HFM). Prerequisite for its long-term application and the envisaged device implantation, all blood contacting surfaces, and the HFM in particular, need to possess complete haemocompatibility. Surface-endothelialization is considered an effective strategy to avoid thrombus formation and subsequent HFM occlusion. While the endothelial cell (EC) monolayer can already be reproducibly established under static conditions, endothelial monolayers of the BHL will need to resist clinically relevant flow conditions. Therefore, we tested if the monolayer on the HFM responds to stepwise increasing flow conditions by the synthesis of basal lamina- and glycocalyx proteins that physiologically facilitate blood flow resistance.

METHODS:ECs were seeded on Fibronectin (HFM-F) and Albumin/Heparin (HFM-H/A) coated HFM and cultivated statically until monolayer confluence. In a customized flow chamber, different flow conditions were applied to adapt the monolayers to the final flow rate (15ml/min). After 24h under flow conditions, monolayers were assessed for changes in confluence and viability, while gene expression analysis (qRT-PCR) and immunofluorescence staining (IF) were conducted for the detection of basal lamina (Collagen-IV, ColIV) and glycocalyx (Syndecan-2, SDC2) proteins. Additionally, changes in the expression level of pro-thrombogenic and pro-inflammatory genes were analyzed. Data were compared with flow-exposed samples deliberately activated with TNFα and statically cultivated samples with or without TNFα activation.

RESULTS:Following initial static cultivation, ColIV generation by the confluent EC-monolayer could be confirmed by IF and qRT-PCR on all tested HFMs. Upon flow exposure, cell detachment was observed on both HFM types, only in areas with shear stresses exceeding set values. The expression of ColIV was neither increased by the HFM coating type, nor the flow application. Gene expression of SDC2 was significantly upregulated after flow only on HFM-F. Expression analysis of activation-relevant genes indicated that ECs on both coatings maintained the non-thrombogenic and non-inflammatory state.

DISCUSSION & CONCLUSIONS:EC monolayers on both HFM are capable to generate a ColIV-containing basal lamina-like matrix already under static conditions. A flow-induced increase in the production of glycocalyx-associated SDC2 was detected only on HFM-F. The current experimental setup provides an eligible platform to detect the flow-mediated generation of further matrix proteins and to develop a flow adaption protocol for the maturation of a matrix that facilitates the long-term resistance of the BHL EC-monolayer towards blood flow.

Keywords: Interfaces - biological, Advanced therapy medicinal products

Bone regeneration with two different bioactive factors incorporated hydroxyapatite microcarrier

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INTRODUCTION:The use of growth factor with a carrier system, represent a promising tool in the tissue engineering. The bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) are the main bioactive factors of which using with ceramic scaffold. The goal of this study was to determine whether hydroxyapatite microcarrier (MC) with dual delivery of osteogenic and angiogenic factor accelerate hard tissue regeneration in regenerative process

METHODS:Three MCs with various sizes were fabricated by emulsification of gelatin and alpha-TCP. The experimental group was divided according to the combination of MC size and growth factors. Rat mesenchymal stem cells (rMSCs) harvested from the bone marrow to investigate in vitro properties. Proliferation of rMSCs and osteogenic differentiation were analyzed. MC with/without growth factors were applied into the standardized, 5-mm diameter defects, which made bilaterally on parietal bone of the rat. The animals were allowed to heal for 8 weeks and samples were harvested and analyzed by micro-computed tomography and histologically

RESULTS:Proliferation of rMSCs was improved on VEGF loaded MC. Osteogenic differentiation indicated dual growth factors delivered MC showed higher osteogenic gene expression, ALP production and calcium deposition. In vivo results revealed statis¬tically significant increase in new bone formation in dual growth factors delivered MC

DISCUSSION & CONCLUSIONS:Dual growth factors administered on calcium phosphate matrix significantly enhanced osteogenic potential, and have potential clinical utility in providing solutions for craniofacial bone defects, with the added benefit of earlier availability

Keywords: Musculoskeletal (inc ligament / tendon / muscle / etc)

Improbable bachelor - Polybenzimidazole as a novel biomaterial for neural tissue engineering

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INTRODUCTION:The central nervous system is a highly electrical active tissue. When affected by disease (neurodegenerative or psychiatric), electrical activity is disrupted, and cell death occurs. Deep brain stimulation (DBS) is a technique that makes use of implantable probes to record electrical activity and stimulate electrically the brain tissue, improving brain performance. While high quality of signal recording and transmission are important requirements for such probes, successful tissue integration with intimate cell-probe interaction will determine the long-term effectiveness of DBS.
Polybenzimidazole (PBI) is an electroconductive polymer that can be doped with strong acids to change its electroconductivity. PBI can be processed into different 3D-structures without the use of carrier polymers. For DBS probes, when compared with metals PBI presents better mechanical properties that determine successful tissue integration.

METHODS:This work evaluates, for the first time, the biocompatibility of PBI's electrospun fibers. Electrospun PBI fibers were doped with organic and inorganic acids, and then characterized with respect to morphology, physico-chemical properties, electroconductivity and biocompatibility following ISO10990 guidelines. In vitro studies with ReN cells were used to determine the applicability of PBI in neural tissue engineering. Changes in morphology, growth rate, and protein and gene expressions were evaluated.

RESULTS:Electrospun fibers of PBI, with diameters within the nanometer range (130-200nm), were obtained. Doping was successful with sulphuric acid and camphorsulphonic acid. However, increasing the electroconductivity was only possible when sulfuric acid was used ($(2.4 \pm 2.2) \times 10\text{-}4$ S/cm). ReN-VM cells growth in a proliferation assay (10 days) did not show major signs of toxicity, where cells interacted with the matrix, morphology was maintained, and a constant cell number increase was observed for all samples tested. Differentiation was also successfully conducted on fibers doped with camphorsulfonic acid (4 days). The obtained cells expressed differentiation gene and protein markers for both neurons and astrocytes.

DISCUSSION & CONCLUSIONS:PBI holds promise in biomedical applications for designing new electroconductive probes for DBS, since neural cells can intimately interact with it and differentiate. Doping with organic acids is also possible and potentially bioactive molecules can be immobilized on PBI structure to fine tune its bioactivity. Overall, our results show PBI is a suitable candidate for electroconductive scaffolds for neural tissue engineering applications [1].

Acknowledgements:The authors thank Fundação para a Ciência e Tecnologia for funding through iBB (UID/BIO/04565/2019), Neuron (PTDC/CTM-CTM/30237/2017), scholarships (PD/BD/114045 2015, SFRH/BD/105771/2014) and PORL 2020 through PRECISE (16394)

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Keywords: Biomaterials, Nanomaterials (inc graphene)

Magnetically sculped laminarin-based hydrogels for future TE applications

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INTRODUCTION:The recent convergence of biomaterials and micro/nanotechnology has enabled the development of smart materials using magnetic nanoparticles (MNPs). Additionally, for such materials to better mimic native tissues, MNPs may be functionalized with specific biochemical signals in order to create featuring channel-like topographies that allow to control the cellular environment[1]. Further encapsulation of MNPs enables the development of larger magnetic materials/particles that could be also used to immobilize a larger number of bioactive molecules such as enzymes. Due to the ability to remotely control in a spatiotemporal manner, functionalized-encapsulated-MNPs can be further seeded on 3D-hydrogels to create a topographical patterning that potentiate cell responses[2]. Among the variety of materials, laminarin, a polysaccharide isolated from brown seaweeds, has been recently proposed as an ideal polymer to produce hydrogels due to its intrinsic low viscosity and high solubility[3]. Here, we intended to create well-defined topographies within laminarin 3D-hydrogel by combining controlled enzymatic digestion with magnetic-field guidance. Relying on the position of this external force different topographies will be engraved.

METHODS:Firstly, MNPs were encapsulated in polycaprolactone (m-PCL) following an electrohydrodynamic atomization protocol[4]. Secondly, laminarin was methacrylated allowing further photo-crosslinking towards the formation of the final laminarin hydrogel (MeLam). Thirdly, laminarinase was immobilized onto m-PCL (mL-PCL) through a previously amino- and glutaraldehyde functionalization and then characterized by FTIR, XRD, TGA, ICP-AES and XPS[5]. Enzymatic activity of mL-PCL was evaluated by DNS method. Under an external magnetic field, mL-PCL were seeded on MeLam (in PBS), which progressively migrated towards the magnet and accumulated at the bottom side, creating a micropatterning within MeLam. Such phenomenon was accessed by confocal laser microscopy and SEM microscopy. The release of the laminarinase by-product glucose was also monitored along time.

RESULTS:Results indicate that particles with a diameter ranging between 20-30 µm, with spherical shape and supermagnetic behaviour were obtained. Moreover, laminarinase was successfully immobilized onto m-PCL, which was corroborated by Bradford protein assay, enabling the engravement of MeLam. Regarding their bioactivity laminarinase functionalized in m-PCL showed long term stability.

DISCUSSION & CONCLUSIONS:By combining magnetic-field guidance and enzymatic digestion, defined 3D-paths were created within MeLam, opening news insights for the creation of complex engineered tissues. Since the main laminarin degradation product is glucose, we envisage that cells cultured within MeLam will benefit from their own nutrition, overcoming the current limitations of scaffold-based approaches.

Acknowledgements:We thank the financial support of ERC-2014-ADG-669858-“ATLAS”, the FCT project “PROMENADE”-PTDC/BTM-MAT/29830/2017 and the PhD scholarship (SFRH/BD/146740/2019).

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[5]-10.1021/bm020074y

Keywords: Hydrogels and injectable systems, Biomechanics / biophysical stimuli and mechanotransduction

Patient-specific biocompatible polymer scaffolds for bone tissue regeneration

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INTRODUCTION:Additive manufacturing technologies (AMT's) like digital light processing stereolithography (DLP-SLA) provide the opportunity to manufacture biocompatible patient specific materials for bone tissue engineering approaches.[1] Highly crosslinked state of the art materials based on (meth)acrylate resins cannot meet the high requirements regarding mechanical properties and biocompatibility. Novel vinyl ester (VE) monomer systems with considerably lower cytotoxicity and polyvinyl alcohol as favorable degradation product, emerge as promising biocompatible precursors for photopolymers.[2–4] The established vinyl ester monomers are suitable for precisely manufactured and customized scaffolds for bone tissue regeneration. Photopolymers show very low toughness in general due to their characteristic polymer architecture. Nevertheless, by using toughness enhancers, high molecular weight co-monomers as well as chain transfer agents, toughness can be increased to ensure fixation of implants by screws.[6]

METHODS:VE-based systems were investigated and compared to (meth)acrylate benchmark systems.[5] Regulation of the polymer network via thiol-ene chemistry leads to increasing reactivity and improved mechanical properties, especially thoughness.[2+6] Further toughness improvement was achieved by including newly synthesized VE's with spacers of variable molecular structures and different additives. In vitro tests with murine fibroblasts were performed to investigate monomer cytocompatibility. Photoreactivity was verified via RT-NIR photorheology.[7] Further dynamic mechanical thermal analysis (DMTA), tensile testing and impact testing provided information about (thermo-)mechanical properties of the crosslinked materials.[6]

RESULTS:Thiol-ene chemistry demonstrated to be a suitable tool to increase resin reactivity and improve the mechanical performance of the photopolymers due to its network regulation capabilities. In order to use DLP-SLA as advanced 3D printing technique a defined inner and outer structure can be obtained with feature resolutions down to the sub-micron range. Preliminary in vivo studies with these scaffolds over 12 weeks showed no inflammation, ongoing biodegradation and excellent vascularization.

DISCUSSION & CONCLUSIONS:Tough 3D printable scaffolds based on VE polymers are well suited as screwable implants for bone tissue regeneration with excellent biocompatibility and biodegradation behavior.

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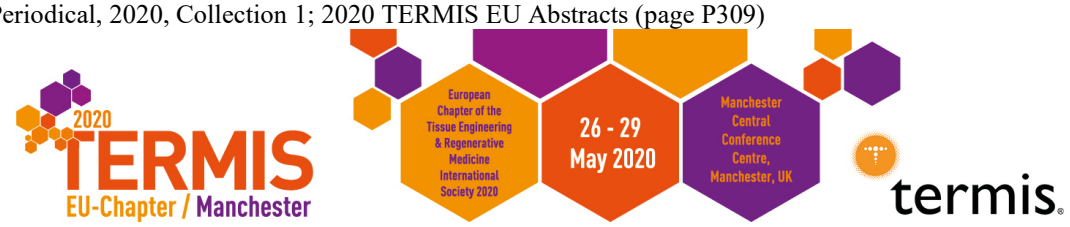
Keywords: Biomaterials, Additive manufacturing



A Critical View on the Mechanical Properties of Biodegradable Thermoplastic Polyurethanes for Vascular Tissue Regeneration

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INTRODUCTION:Biodegradable materials as scaffolds for tissue engineering are emerging for many uses, including small-diameter vascular grafts.[1] It was often observed that state-of-the-art materials, esp. thermoplasts, exhibit excellent biodegradation, however, mechanical stability is not sufficient for the designated application. In the last decade we introduced a variety of novel hard block degradable thermoplastic polyurethanes (TPUs) by incorporating cleavable chain extenders into the molecular structure of the polymers.[2–4] The comparison of all these polymeric materials has led to new insights on the mechanical properties of biodegradable thermoplasts, which laid the foundation for future research in this field.

METHODS:TPUs based on novel cleavable chain extenders were prepared by the prepolymer method[2–4] and characterized by mechanical testing, degradation experiments, and biocompatibility assays. Electrospinning was employed to manufacture highly porous tubes with diameters between 1.5 and 2.0 mm. The obtained conduits were tested in vitro for their biocompatibility, their biomechanical behavior and subsequently also in vivo.

RESULTS:In this study, we compared different modifications of hard block degradable TPUs with emphasis on their degradation and mechanical characteristics. It is widely agreed upon that the hard block quality, which is highly influenced by the choice of chain extender and isocyanate, determines the mechanical properties of the materials. Effects resulting from these insights were considered for the chain extender designs in this study. To date, the concepts for degradability predominantly rely on ester cleavage in soft blocks. We integrated this function into the chain extenders and expanded the range of degradable moieties while maintaining their ability to form strong microcrystalline domains. By variation of the components and their ratio for the polymer synthesis, a wide range of different properties were observed, which justifies the systematic approach of the study.

DISCUSSION & CONCLUSIONS:In this study, we found that a well-designed combination of prepolymers and chain extenders results in hard block degradable TPUs with excellent mechanical stability for small-diameter vascular grafts, superior biocompatibility in vitro, and encouraging performance in vivo.

ACKNOWLEDGEMENTS:The financial support of Austrian Wirtschaftsservice (AWS) (P1405063), the Techniker-Cercle, and the Ludwig Boltzmann Society is kindly acknowledged.

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Keywords: Cardiovascular, Biomaterials

Optimized synthesis of photocrosslinkable hyaluronan for efficient hydrogel formation

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INTRODUCTION:Hyaluronan (HA) is a naturally occurring polysaccharide, which can be chemically modified for many tissue engineering applications, while being enzymatically cleared ""in vivo"" [1]. We have previously described basic synthesis of tyramine substituted HA (HATA), which afforded covalently cross-linked hydrogels via enzymatic crosslinking [2]. Now we describe an optimized synthetic protocol for HATA with an increased degree of substitution (DS) and wide range of molecular weight (Mw). This novel material provides a fast gelation process as well as improved viscoelastic properties of the photocrosslinked hydrogels.

METHODS:HATA was synthesized by reductive amination with oxidized HA [2] and tyramine with aminohexanoic acid spacer [3]. The DS was determined by NMR and Mw was measured by size-exclusion chromatography. The hydrogels were prepared from 0.9 % NaCl solutions (1-2% w/v) with riboflavin as a photosensitizer (10-100 µg/ml) using LED source (95-105 mW/cm2 at 445 nm). The gelation kinetics and viscoelastic properties of the hydrogels were analysed by DHR rheometer (TA Instruments) in 3 repetitions with ±10% variability.

RESULTS:The most crucial parameter in optimization was pH. It must be kept in acidic range in order to achieve an efficient reduction step. The optimal reactions conditions, which gave rise to almost quantitative conversion of a starting aldehyde (≥95%) without a significant polymer degradation (max. 15%) were following: 0.5 equivalent of amine per HA dimer, AcOH (pH = 4.8-5.2), 1 equivalent of picoline borane complex per HA dimer, 1+7 h, 50 °C, 25% DMSO. A series of derivatives with DS ≥ 6% and Mw = 250-1100 kDa was prepared according to this protocol. Several derivatives differing in DS and Mw were subjected to photocrosslinking. The comparison of 1% HATA gels with DS = 2.6%, 7.1%, and Mw = 281 kDa, 974 kDa showed a shortened gelation time to one third (tg = 3s) and increased elastic modulus by 230% (G' = 580 Pa at 180 s).

DISCUSSION & CONCLUSIONS:This work described an optimized synthetic protocol to photocurable HA derivative with increased DS and Mw for rapid gelation and outstanding viscoelastic properties of the photocrosslinked hydrogels.

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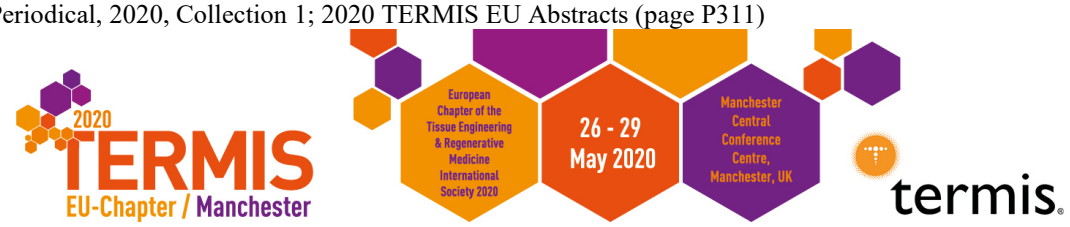
Keywords: Hydrogels and injectable systems, Biomaterials



Rapid prototyping 3D parallelized microfluidic droplet generators using stereolithographic printing for high throughput micromaterial fabrication

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INTRODUCTION:Microfluidic droplet generation is highly suitable for numerous applications including (single) cell analysis, cell therapies, pharmacological screenings, emulsion chemistry, and micromaterial fabrication. However, upscaling of microfluidic processes to clinically and industrially relevant quantities remains challenging, mainly due to the two dimensional (2D) or 2.5D nature of conventional fabrication methods. Moreover, current parallelization strategies often fail to preserve monodisperse droplet production. To overcome these limitations and enable the large-scale generation of monodisperse microdroplets, we used computational fluid dynamics simulations in combination with high-resolution stereolithographic printing to engineer 3D parallelized microfluidic droplet generators. We hypothesize that these 3D parallelized microfluidic devices can be compatible with the upscaled production of microdroplets of various sizes, compositions, and complexities. Specifically, we explored the production of cell-laden hollow micromaterials, which offer 3D physically confined microenvironments to cells. This strategy efficiently allowed for the generation of cellular spheroids and organoids.

METHODS:Computer-aided design software and stereolithographic printing of PIC100 resin were used to manufacture a flow focusing droplet generator with a 100 µm wide nozzle. 3D reconstructed microcomputed tomography analysis was performed, and the degree of fidelity was evaluated. The functionality of the 3D printed droplet generators was demonstrated by producing fluorescently labeled water-in-oil emulsions, which was used to create stable aqueous microdroplets. To achieve 3D parallelization, stacked microfluidic droplet generators were arranged in a radial manner. To demonstrate the compatibility of the production of micromaterials, mesenchymal stem cells (MSCs) were encapsulated into monodisperse microcapsules composed of dextran-tyramine conjugate. The microencapsulated cell's viability, micro-spheroid formation, and function was investigated.

RESULTS:Microdevices were successfully fabricated using stereolithography, which enabled the manufacturing of hollow channels with dimensions as small as 50 µm. The microdevices could be operated up to at least 4bar without breaking, structural damage, deformation of channels, or leakage of the on-chip printed Luer-Lok type connectors. The printed microdevices enabled the production of water-in-oil emulsions, as well as polymer containing droplets that acted as templates for both solid and core-shell hydrogel micromaterials. The MSCs encapsulated in monodisperse dextran microcapsules showed high viability rates (>95%). Moreover, controlled formation of stem cell spheroids was achieved.

DISCUSSION & CONCLUSIONS:In summary, we demonstrated that stereolithography fabricated microfluidic devices allow for the parallelization of droplet generators in a simple yet effective manner by enabling the realization of (complex) 3D designs. Thus, stereolithographic printing of 3D parallelized microfluidic droplet generators has shown significant potential to simplify and accelerate micromaterial production for biomedical, cosmetics, food, and pharmacological applications.

Keywords: 3D printing and bioprinting, Additive manufacturing

Bringing Safe and Standardized Cell Therapies to Industrialized Processing for Burns and Wounds

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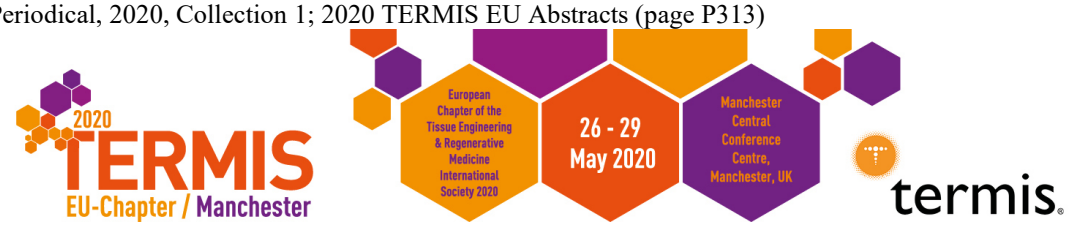
INTRODUCTION:Cultured primary progenitor cell types are worthy therapeutic candidates for regenerative medicine. Clinical translation, industrial transposition and commercial implementation of products based on such cell sources are mainly hindered by economic and technical barriers, as well as stringent regulatory requirements. Applied research in allogenic cellular therapies in the Lausanne University Hospital (CHUV) focuses on cell-source selection technique optimization. The use of fetal progenitor cell sources in Switzerland is regulated through a Federal Transplantation Program and an associated Fetal Biobank. Clinical applications of cultured primary progenitor dermal fibroblasts have been optimized in the CHUV since the 1990s. These novel cell sources have been developed into “Progenitor Biological Bandages” for pediatric burn patients and adults presenting chronic wounds.

METHODS:A single organ donation (2009) enabled the establishment of a standardized cell source for clinical and industrial developments to date. Non-enzymatically isolated primary dermal progenitor fibroblasts (FE002-SK2 cell type) served for the establishment of a clinical-grade Parental Cell Bank, based on a patented method.

RESULTS:Optimized bioprocessing methodology for the FE002-SK2 cell type has demonstrated that extensive and consistent progenitor cell banks can be established. Most importantly, highly successful industrial transposition and up-scaling of biobanking enabled the establishment of tiered Master and Working Cell Banks using Good Manufacturing Practices. Successive and successful transfers of technology, know-how and materials to different countries around the world have been performed. Extensive developments based on the FE002-SK2 cell source have, to date, led to clinical trials for burns and wound dressing (pending in Switzerland; approved in Japan, Taiwan, USA; ongoing in Japan, Taiwan).

DISCUSSION & CONCLUSIONS:The Swiss Fetal Transplantation Program and over 3 decades of pioneer clinical experience in the CHUV Burn Center constitute concrete indicators that primary progenitor dermal fibroblasts should be considered as flagships in the domain of wound healing and regenerative medicine in general. Indeed, one single organ donation potentially enables millions of patients to benefit from high-quality regenerative therapies, while guaranteeing optimal safety and state-of-the-art therapeutic efficiency. This work presents a technical and translational overview of the described progenitor cell technology harnessed in Switzerland as a cellular therapy for treatment of burns and wounds around the globe.

Keywords: Cell therapy, Scaling up & manufacturing



MECHANOCULTURE – Applications of dynamic pressure in manufacturing cell therapies

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INTRODUCTION:Mechanical forces regulate fate decisions in mesenchymal stem cells (MSCs) and musculoskeletal cells such as chondrocytes, yet these forces are not controlled during scale up manufacturing of therapeutic cells. We have developed a pulsed pneumatic system to apply dynamic hydrostatic pressures to cells grown in high-throughput manufacturing platforms such as spinner flasks. By employing the under-used gas phase of the culture environment, our objectives were to induce mechanotransduction in the cells in order to improve the effectiveness and consistency of bioreactor-grown stem cells and chondrocytes.

METHODS:We developed a series of bioreactors using commercially available components and adapted these to our application using 3D printing. We constructed two devices: the J2 bioreactor which is an evolution of our previously published design (Henstock et al, 2013; Reinwald et al, 2015) and uses a compressor and valve manifold to deliver pressures of up to 300kPa at 1Hz. Our second bioreactor, the H3 uses a simple motorized piston to compress the gas inside the culture environment. In each bioreactor, we periodically subjected hMSC and chondrocytes in hydrogel constructs to dynamic pressures over the culture time and monitored the effects on proliferation, phenotype and extracellular matrix production.
>
RESULTS:Both bioreactors achieved their design pressures and maintained sterile cultures over several weeks. Example results show that chondrocytes cultured in alginate hydrogel microspheres increased their ECM production and proliferation rate after just 5 days exposure to dynamic pressures. Human MSCs exhibited increases in paracrine signalling, motility and actin remodelling, but did not differentiate at low pressures.
>
DISCUSSION & CONCLUSIONS:Mechanical stimulation is lacking in most scale up manufacturing platforms, yet we have shown that by simply pulse-compressing the gas phase in the culture vessel, pneumatic pressure is transduced into hydrostatic pressure in the media which is detected by cells as a form of compressive loading – recreating the forces that cells would experience in their native environment during exercise. We have therefore developed a novel ‘mechano-culture’ system that can be easily added to existing scale-up manufacturing platforms to pulse-compress the gas phase and deliver regulatory mechanical stimulation to cells in culture which helps maintain their phenotype during expansion.

ACKNOWLEDGEMENTS:This work was supported by the Wellcome Trust, Royal Society and through funding from the Institute of Ageing and Chronic Disease, University of Liverpool. We would like to thank Ma Ke (UoL) for his work on developing the software for the J2 bioreactor.

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Keywords: Bioreactors, Scaling up & manufacturing

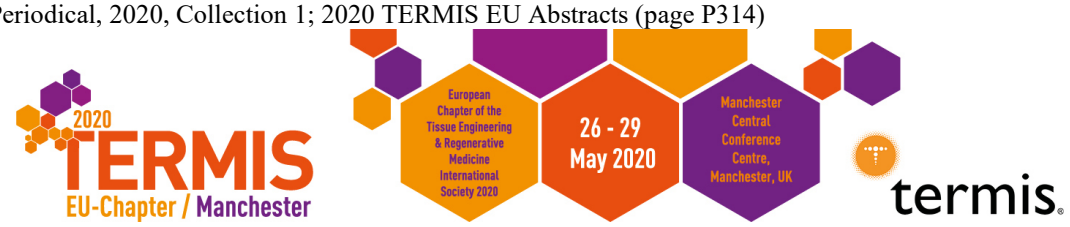

Image-based Cellular State Monitoring for Biopharmaceutical Production Cells

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INTRODUCTION:The bio-pharmaceuticals, such as antibodies and protein-based products, are the next-generation medicinal products which ultimately apply the cell culture technologies. Although they now promise for higher therapeutic effect and lower side effects, their manufacturing technology is much complicated than the conventional chemistry-based products. Since the products' efficacy, safety, and quality always greatly rely on their production cells, it is essential to control their quality in every process level in their manufacturing. "How to maintain cell's quality for their product lifecycle" and "how to select suitable cells for further manufacturing consistency" is the recent requirements in cell culture engineers. From such aspect of stabling technology for cell culture and production, image-based analysis can offer real-time information to feed back for the culture control and optimization. Our group has been investigating the use of microscopic images to non-invasively assess, record, and monitor cell's quality during the cell culture process in manufacturing [1, 2]. In this work, we have expanded our method to evaluate the Chinese hamster ovary (CHO) cells and their delicate differences.

METHODS:CHO cell lines with or without the production, and their metabolism regulator compounds were used to set different quality conditions. For their morphological analysis, BioStation CT (Nikon) were used for punctual and continuous imaging. Autofocusing condition was optimized, and all the time-course image data were analyzed by our own image processing algorithms using Python. Extracting multiple morphological parameters of time-course, the quality differences were analyzed and visualized by clustering and machine learning classifiers.

RESULTS:Our image analysis of morphological parameter extraction succeeded in extracting explainable morphological features not only from the adhesive CHOs but also suspended CHOs, to discriminate their production performances and metabolisms.

DISCUSSION & CONCLUSIONS:By our data, we found that even with the suspended cells, our morphological analysis succeeded in analyzing the delicate differences in CHOs, therefore can be applied to other suspended type cells in cell therapeutic products advancing in the recent regenerative therapies.

Acknowledgements:Authors are grateful for financial support from Medical Research and Development Programs from the Japan Agency for Medical Research and Development, AMED.

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Keywords: Biologics and growth factors, Enabling technologies

A Novel Polyurethane-based Composite Cultured Skin and a Bespoke Bioreactor in a Porcine Wound Model

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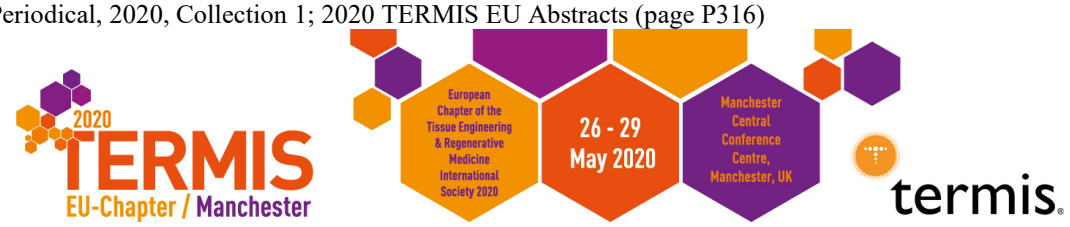
INTRODUCTION:The management of extensive full-thickness burns can be facilitated by a two-stage strategy to replace the need for skin grafting. The first stage uses a biodegradable polyurethane scaffold that temporises the wound. The second is a laboratory based composite cultured skin (CCS) that utilises the same polyurethane-base and comprises collagen-producing fibroblasts and epidermal keratinocytes to assist in final wound closure. Both have undergone pre-testing regimes from in vitro and in vivo animal studies in small wounds. However, to produce enough cultured skin for an average adult; a specialised bioreactor that can produce sizeable CCS is required and reported here in a large wound animal model.

METHODS:Three Large White x Landrace pigs were used; two wounds (24.5cmx12cm) were created and a biodegradable dermal scaffold implanted. A split skin graft was taken as control, covering ~1/3 of a wound. A biopsy was harvested for culture and expansion of autologous fibroblasts and keratinocytes. For CCS setup, 1mm polyurethane foam was pre-soaked in plasma. In the bioreactor cassette fibroblasts were seeded onto the matrix with thrombin until keratinocytes were seeded. The CCSs were applied after scaffold delamination and dermabrasion. Punch biopsies, transepidermal water loss (TEWL) readings and wound measurements were performed to indicate wound healing and skin barrier function.

RESULTS:The wounds displayed minimal contraction (average 4.5%) prior to CCS transplantation. Two of the skin grafts showed 100% take by day 11 and primary islands of clinical epithelium noted on two of the three CCS. Engraftment was confirmed by histological analysis. Complete healing occurred at day 53 post-CCS transplantation with wound stabilisation by day 66. TEWL of CCS approximated normal skin (average 11.9g/m²h) indicating definitive wound closure.

DISCUSSION & CONCLUSIONS:Four of the six wounds demonstrated engraftment producing a robust, stratified epithelium analogous to normal skin. Generating sizeable pieces of CCS is feasible using the bioreactor to heal a large wound. This translational research has now been used on a 95% burns patient, who was transferred to rehabilitation 8 months post injury.

Keywords: Wound healing, Bioreactors



Cell technologies for the full thickness grafting of the face

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INTRODUCTION:At present, the issue of skin recovery after deep and vast burns remains vital, even though the surgical approaches to treatment of patients with burns have been improved. Deep burns of the face are not only a severe burn injury, but also psychological injury. The purpose of our work was to develop of new technical methods for performing of facioplasty with full thickness autografts.

METHODS:Since 2010, in the SRI-Regional hospital No. 1 one full thickness autograft method has been used for deep face burns. Since 2017, the autologous fibroblasts have been used for the skin facioplasty of one full thickness autograft. Human dermal fibroblast were obtained from GMP laboratory. The fibroblasts were isolated and cultivated following the standard operating procedures and was tested for quality control before use. The local ethical committee approved this study and all patients were informed and signed informed consent.

RESULTS:Since 2010, 6 transplant operations of one full-layer autograft have been performed in case of a total burn of the face and cicatricial deformities, in which a positive cosmetic result and the absence of indications for reconstructive operations were noted in the long term. However, the engraftment of a full-layer autograft occurred slowly, up to 14-17 days, and was associated with the slow germination of vessels in the graft and its adaptation. To improve adaptation, we used autologous fibroblasts in 5 cases of total facioplasty. The choice of autologous fibroblasts was due to the fact that the use of own cells eliminates the risk of transmission of blood-borne infections, and creates a favorable microenvironment for graft engraftment. The results of our work showed that autologous fibroblasts provide faster adaptation of the autograft. There was no signs of fibrous tissue with a long period of observation.

DISCUSSION & CONCLUSIONS:The method allows to create conditions for the rapid engraftment and adaptation of a whole full-layer free skin autograft to a granulating wound after its excision as a result of the use of autologous fibroblasts. Performing autoplasty with a full-layer skin autograft using autofibroblasts reduces the risk of developing fibrotic tissue in the long-term postoperative period. The improvement of facial plastic surgery methods allows achieving maximum cosmetic results of treatment.

Keywords: Wound healing, Cell therapy

Towards a more reliable skin fabrication process: A quantitative comparison study of in vitro skin equivalents

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INTRODUCTION:In vitro skin equivalents, built up of different cell types of the human skin, can mimic the native tissue and are thus used for the identification of disease triggers and drug testing. However, a widespread issue is their high intra- and inter-batch variability which is a consequence of their unstandardized production. Additionally, the methods which are used to validate the quality of skin equivalents are vastly based on subjective evaluations, and quantitative methods are rarely used. This fact, and the fact that the actual variability of in vitro skin equivalents per se is not known, limits the reliable comparison of skin equivalents between experiments and between various groups.

METHODS:In this study, we have defined a set of quantitative evaluation metrics in skin equivalents to replace the subjective evaluation with actual numeric attributes. These metrics are applicable on intermediate results during the production process as well as the final skin equivalent. Using the established metrics, we have evaluated two of the most commonly used types of skin models. Therefore, cells were isolated from healthy human skin biopsies and used for the production of scaffold-free and scaffold-based skin equivalents consisting of fibroblasts and keratinocytes.

RESULTS:We show the resemblance of the final skin equivalents to native skin, the intra-and inter-protocol variability and the intra-and inter-donor variability. Further, we have identified several factors that result in inhomogeneity within a single replicate as well as in variability between various replicates.

DISCUSSION & CONCLUSIONS:The quantitative evaluation provides a reliable method to compare results between different experiments. Based on the evaluation of intermediate fabrication steps, it further enables a profound identification of factors of variation. Being aware of these factors is a first step towards the development of standardised and reproducible protocols for skin equivalents with reduced variation.

Keywords: Disease models, Scaling up & manufacturing

Generation of a Skin Advanced Therapies Medicinal Product with Antibacterial Activity

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INTRODUCTION:Several bioartificial skin substitutes have been developed to the date. However, one of the main limitations of this technology is the concomitant bacterial infection affecting most severely burnt patients, especially by Pseudomonas aeruginosa (PAO). The objective of this work is to develop novel human bioartificial skin substitutes with antibacterial properties that could improve the results of the treatment in burnt patients with PAO contamination.

METHODS:Human bioartificial skin substitutes were generated using fibrin-agarose biomaterials. To provide the substitutes with antibacterial properties, lipid nanoparticles loaded with amikacin were incorporated to the biomaterial at increasing concentrations (10, 100 and 300 mg/l). Then, these substitutes were set on PAO cultures on agar-LB plates and bacterial growth was assessed after 24h of culture at 37ºC by measuring the inhibition area in which bacteria are not able to grow around the tissue. Control skin substitutes containing mock empty nanoparticles and without nanoparticles were used. Three independent tissue samples were evaluated in each case.

RESULTS:Bioartificial human skin substitutes containing amikacin-loaded nanoparticles showed PAO growth inhibition in a dose-dependent manner. First, PAO cultured in the presence of the substitutes without nanoparticles (controls) were able to grow and proliferate in 99.7% of the culture plate surface. Then, bacteria cultured in plates with substitutes containing 100 and 300 mg/l of amikacin-loaded nanoparticles displayed a proliferation area of 43.2% and 15.0% of the plate surface, respectively. Control samples containing empty nanoparticles showed bacterial growth in 99.6% of the plate surface.

DISCUSSION & CONCLUSIONS:These results suggest that human skin substitutes containing nanoparticles loaded with specific anti-PAO antibiotics exert anti-bacterial effects, especially at the concentration of 300 mg/l. This effect could be very useful to prevent PAO contamination in patients with severe skin burns, which is one of the major causes of treatment failure in these patients.

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Keywords: Biomaterials, Skin

In vitro study of different biomaterials combined with fibrin for the manufacture of human Tissue Engineered Skin Substitutes

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INTRODUCTION:Biomaterials are the backbones of any human Tissue Engineered Skin Substitute (hTESS), because provide a platform for cells during the healing process. The structure, morphology, surface topography and mechanical elasticity of scaffolds play a crucial role in cell metabolic activities[1]. In addition, an adequate dermal matrix, emerged as a necessity to find a useful transplantation technique which allows the interaction between human fibroblasts and keratinocytes[2]. Our group has experience in fibrin-hyaluronic acid biomaterial, however, other biomaterials that possess different characteristics have been investigated; collagen, fibronectin or laminin and the decision to use one or another, is important to achieve successful results.

METHODS:12 bilayered hTESSs (human fibroblasts and keratinocytes) were fabricated in wells of 6-insert-well-plate. Scaffolds were a combination of fibrin and different concentrations of several biomaterials (hyaluronic acid, collagen, fibronectin, s-peptide and laminin). Cells were isolated from human skin biopsies and cultured under standard conditions until final recovery. 150,000 human fibroblasts and 300,000 human keratinocytes were added to each hTESSs and maintained in culture for 1 week, resembling our Good Manufacturing Practice's protocol. After this period, hTESSs were analysed and compared using techniques such as PrestoBlueTM, LIVE/DEAD® and immunohistochemistry.

RESULTS:All biomaterials and concentrations were biocompatible with cells; results of PrestoBlueTM assay revealed that cell reducing power was higher in those hTESSs constituted of collagen (67 ± 1.02% and 65 ± 1.43%). Biocompatibility was also determined by LIVE/DEAD® cell viability assay corroborating these results. hTESSs had a correct morphological structure; and expression of cytokeratins and collagen IV were observed.

DISCUSSION & CONCLUSIONS:Despite of the fact that the number of hTESSs of each type was reduced, and complementary studies are required; such as rheological characterization and in vivo analysis; we can determine that the combination of fibrin with these biomaterials could be an alternative to our hTESSs based on fibrin-hyaluronic acid. Best results were observed using collagen, however, depending of the cutaneous pathology could be interested to use other biomaterials, to achieve the most successful treatment for patients.

ACKNOWLEDGEMENTS:Supported by Carlos III Health Institute of Spain (PI-17/02083 and PhD fellowship FI18/00269 – Álvaro Sierra-Sánchez) and Andalusian Regional Government (SAS PI-0458-2016).

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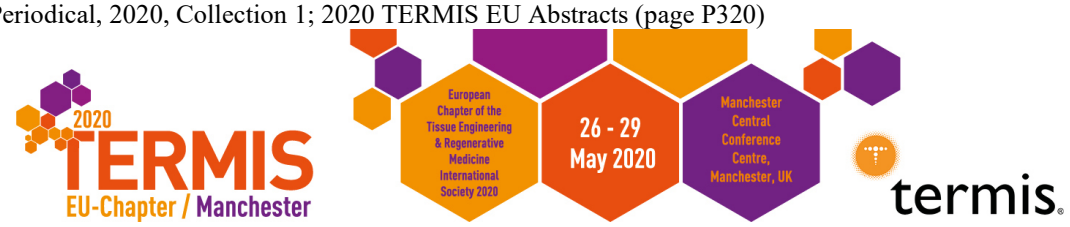
Keywords: Biomaterials, Advanced therapy medicinal products



Regeneration of Melanocytes and Langerhans Cells in a Human Artificial Skin by Tissue Engineering

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INTRODUCTION:Development of biomimetic tissue engineered skin models allowed the efficient treatment of patients with severe skin damage. In this regard, we previously generated a biocompatible model of human skin using nanostructured fibrin-agarose biomaterials with autologous dermal fibroblasts and epidermal keratinocytes called UGRSKIN (1,2). This model was efficiently implanted in patients with severe burn injuries. The objective of the present work is to analyze the development of two extrinsic cell types in the skin grafted in the patients as markers of efficient biointegration and differentiation.

METHODS:To generate the UGRSKIN model, a dermal substitute was generated by combining a fibrin-agarose biomaterial with autologous dermal fibroblasts isolated from skin biopsies. After gelation, autologous keratinocytes were seeded on top and kept in culture for 3 weeks to induce the development of an epithelium-like layer. This tissue engineered skin was grafted autologously in patients affected by severe burns and samples of regenerated skin were collected 1, 2 and 3 months after grafting. Topographical expression of MELAN-A and CD1a molecules was performed by immunostaining. Untreated native human skin was used as control.

RESULTS:We first found that the skin was properly integrated in the host from the first month of follow-up, with the development of a stratified epithelium on top of a dermal substitute that closely resembled native skin. Cells showing positive signal for MELAN-A were found at the basal stratum of the epidermis after 2 months, whereas CD1a-positive dendritic-shaped cells were detected at the spinosum stratum after 1 month of follow-up. Expression of both markers was positive at 3 months.

DISCUSSION & CONCLUSIONS:The bioengineered human artificial skin by tissue engineering allowed the development of an epidermal-like tissue that closely resembled native epidermis from the first month. In addition, melanocyte and Langerhans cells were found and properly differentiated in the epithelium of the skin, suggesting that the regenerated epithelium could be properly differentiated and could properly exert the functions of the epidermis in the patient.

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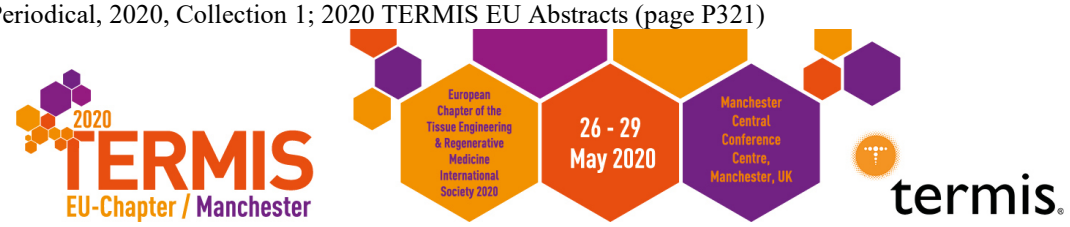
Keywords: Skin,



Human Periosteum-Derived Cells intended for bone tissue engineering show different properties depending on their tissue origin

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INTRODUCTION:The development of alternatives for autologous bone grafts is a major focus in bone tissue engineering. For cell-based strategies, skeletal stem and progenitor cells (SSPCs) are expected to play a significant role in the development of alternatives and are being intensively studied. SSPCs can, among others, be obtained from bone marrow, adipose tissue, dental pulp and periosteum. In view of the development of tissue engineered implants for craniofacial applications, we investigated human periosteum-derived cells (hPDCs) isolated from maxilla and mandible (craniofacial hPDCs) and compared these to the more standardized tibia-derived hPDCs. Although the craniofacial hPDCs are anatomically in close proximity, these do show significant differences in embryological origin and bone tissue properties.

METHODS:Cells from all three sources were cultured up to 12 weeks. Proliferative capacity was quantified by calculating the cumulative population doublings and doubling time. Further characterization was performed by senescence-, MTT- and EdU-stainings at weeks 6, 9 and 12. FACS was performed for essential markers and in vitro trilineage differentiation was studied. RNA-sequencing of hPDCs was performed to explore the different gene expression profiles, which were subsequently validated by RT-qPCR for a panel of 30 genes. Finally, to assess in vivo bone formation, hPDCs were seeded on a NuOss scaffold and implanted ectopically in mice. Mineralization was evaluated after 8 weeks by nanoCT-scanning and histological stainings.

RESULTS:We demonstrated that the craniofacial hPDCs fulfill criteria of SSPCs, those being continuous self-renewal and trilineage differentiation ability towards chondrogenic, osteogenic and adipogenic cells. Both types of craniofacial hPDCs showed similar proliferation rates, comparable to tibial hPDCs. Cells from all three origins were found to be negative for hematopoietic lineage markers and positive for markers linked to SSPCs (CD73, CD90 and CD105). RNA-seq analysis identified 780, 841 and 438 differentially expressed genes between respectively tibial and maxillary, mandibular versus tibial, and mandibular versus maxillary hPDCs. These differentially expressed genes, such as the HOX-genes, DLX-genes and SOX9, are involved in skeletal and cartilage development, extracellular matrix organization, ossification and other related biological processes and molecular pathways. In vivo bone mineralization was observed in constructs containing tibial and mandibular hPDCs.

DISCUSSION & CONCLUSIONS:Taken together, we show that craniofacial hPDCs are a novel cell source for bone tissue engineering in addition to tibial hPDCs. In particular, the mandible-derived cells display -both in vitro and in vivo- chondrogenic and osteogenic differentiation ability that will allow exploitation of both intramembranous and endochondral ossification pathways in craniofacial bone tissue engineering.

Keywords: Developmental biology

Senescence of epithelial Lgr6+ progenitors suggests functional stem cell exhaustion in COPD

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INTRODUCTION:Chronic obstructive pulmonary disease (COPD) is currently the fourth prominent reason for morbidity and mortality, accounting for more than 3 million deaths per year worldwide. Recent studies demonstrated that COPD development is strongly connected to cellular senescence, a process which may impact on functionality of epithelial lung progenitor cells.
In previous publications, we reported the existence of a LGR6-positive population that is responsible for maintenance of epithelial cells in the bronchioalveolar compartment. Here we investigate the status of epithelial LGR6+ cells in COPD.

METHODS:(1) Human lung samples were obtained from patients with undergoing lung transplantation and healthy control samples was collected from cancer patients (tumor-free areas) and donors' material. Sections of 5µm were cut from snap frozen tissues and stained for senescence associated β-Galactosidase (SA-βGal) activity as a marker for cell senescence. In parallel, paraffin embedded sections were stained for detection in immunofluorescence (IF) of LGR6 and lung epithelial populations such as CK5+ basal, CC-10+ Club and SP-C+ alveolar type II cells.
(2) Human lung specimens were dissociated and resuspended in a mix of collagenase/dispase containing DMEM/F12 solution. After mesh and removal of red blood cells, single cell suspensions were fixed, permeabilized and fluorescently labelled for stem and lung markers. A fraction of isolated cells was also tested for SA-βGal activity. Samples were analyzed with flow cytometer.

RESULTS:IF analysis on human lung tissues shows co-expression of LGR6 with CC-10 and SP-C. Increased LGR6 expression was reported in both cellular types, when compared to healthy controls. Investigation of single cell suspensions via flow cytometry revealed an increased SA-βGal activity in LGR6+ cells obtained from COPD cases, which was further explored in immunohistochemical stainings.

DISCUSSION & CONCLUSIONS:Our analysis confirmed expression of LGR6 in distinct populations of epithelial progenitor cells in the lung, supporting the role of LGR6+ cells as common lung stem cell ancestor. Moreover, the increased SA-βGal activity and LGR6 expression points toward an impairment in these epithelial progenitors, accompanied also by evident morphological changes. Altogether, our findings represent a first evidence of exhaustion of the LGR6+ population in COPD.

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Keywords: Stem cells - general, Other

Mechanomics- and Polymeric Materials-Engineering to Regulate Stem Cell Structure & Function

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INTRODUCTION:Stem cells (SCs) provide the basic means for cell population growth, as well as the raw materials for tissue genesis, growth and adaptation during prenatal development and postnatal healing. Surprisingly little is known about SCs' emergent complexity, e.g. mechanoadaptation of the cytoskeleton, transition between adherent and motile states, manufacture and assembly of structural proteins and tissue architectures by cells.[1-3] Integrative approaches are needed to (i) understand and modulate complex behaviours that emerge from single cells and multicellular constructs [3] and (ii) apply this understanding for engineering of mechanochemical stimuli-responsive medical devices and therapies that harness the cells' own healing potential.

METHODS:To study expression of structural proteins by SCs, live SC mechanoadaptation, as well as emergent architectures of tissue neogenesis, two complementary protocols were developed and tested.[3] These protocols enabled live imaging of cell mechanoadaptation (including changes in actin and tubulin as well as cell and nucleus shape and volume), in adherent and motile SCs, during exposure to controlled mechanical loads. In parallel, a simple one pot miniemulsion polymerisation technique4 was implemented to encapsulate drugs (cytoskeleton-modulating agents) within 'hollow' core carriers [Reagents: polymer (PMMA), ultrahydrophobe (hexadecane), initiator (AIBN), surfactant (SDS), disulfide crosslinker (DSDMA)] designed for release of drugs via reduction of disulphide bonds by high intracellular concentration of glutathione.

RESULTS:Pilot hollow core drug carrier studies demonstrate feasibility to formulate and control encapsulation and release of Paclitaxel, a chemotherapeutic agent that prevents disassembly of microtubules. Live imaging of cells treated with controlled release of Paclitaxel reveals spatio-temporal changes in cell mechanoadaptation, observable as changes in cell and nucleus shape and volume, as well as actin (tension resisting) and tubulin (compression resisting) structure and dynamics, predicting adhesion and motility.

DISCUSSION & CONCLUSIONS:This ongoing R&D program is expected to provide a novel, integrative perspective on the mechanisms underpinning SCs' capacity to adapt and evolve to dynamic mechanical signals intrinsic to life. The work provides novel insights into mechanomics- and polymeric materials-engineering to regulate stem cell structure and function. By combining current therapeutic agents with mechanomics engineering and polymer materials design and engineering, we expect this program to broaden targets and approaches for regenerative medicine.

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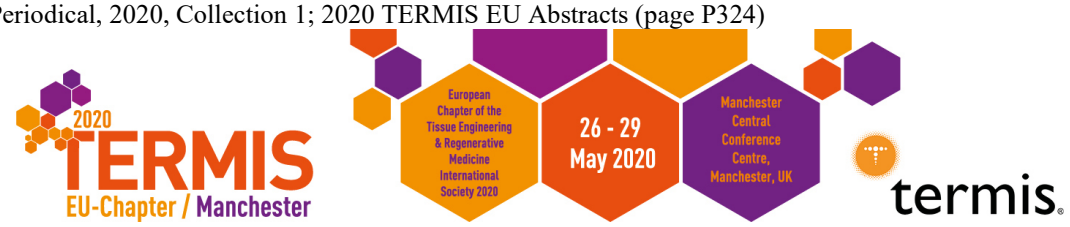
Keywords: Omics / bioinformatics and systems biology, Drug delivery



Osteogenic potential of Adipose-Derived versus Bone Marrow Mesenchymal Stem Cells in an Ectopic Model

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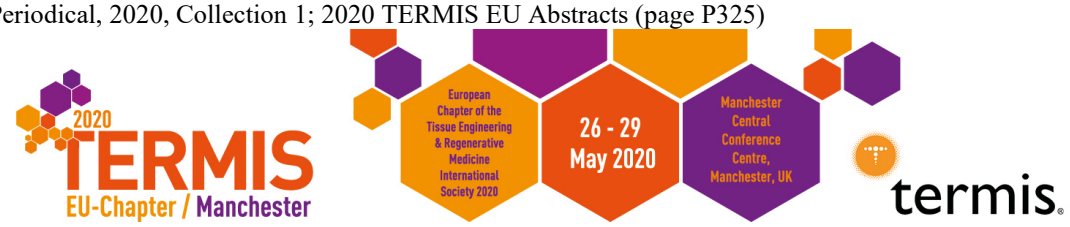
INTRODUCTION:Adipose-derived stem cells (ASC) have been proposed as an alternative to bone marrow mesenchymal stem cells (BMSC) for many applications. Studies comparing human ASC and BMSC for bone tissue engineering have reported inconsistent results regarding superiority of stem cells from one source over the other. Human ASC were frequently compared to BMSC obtained from different individuals. Consequently, differences between the individuals might have influenced the properties of these cells and contributed to this inconsistency in the results. Therefore, we aimed to compare the in vitro proliferation and differentiation potential of donor-matched human ASC and BMSC and to test their ectopic in vivo osteogenic potential in immunodeficient mice.

METHODS:Human ASC and BMSC were first characterized and then seeded into poly (L-lactide-co-ε-caprolactone) scaffolds. Cell proliferation and multi-differentiation were tested in vitro. This was followed by random subcutaneous implantation of Scaffolds with ASC or BMSC, and scaffolds without cells (control) in the dorsum of NOD/SCID mice. Formation of mineralized tissue was evaluated at the gene level, using micro computed tomography (µCT) and histologically.

RESULTS:ASC and BMSC had morphological and immunophenotypical characteristics of the stem cells. ASC and BMSC attached on the scaffolds and showed similar proliferation rate. ASC and BMSC also demonstrated comparable in vitro differentiation capacity into osteogenic, adipogenic and chondrogenic lineages. After 2 weeks in vivo, scaffolds with BMSC and scaffolds with ASC showed similar expression of the osteogenesis-related gene alkaline phosphatase, but expression of the genes osterix, osteopontin and osteocalcin was higher in scaffolds with BMSC. After 10 weeks, formation of mineralized structure was not observed by µCT and histological examination, but more organized collagenous matrix was formed in scaffolds with BMSC than with ASC.

DISCUSSION & CONCLUSIONS:ASC and BMSC had comparable in vitro proliferation and multi-lineage differentiation potential. Using the ectopic model, the osteogenic capacity of BMSC at the gene level was greater than ASC, but this was not translated further into formation of bone-like structure in vivo. Since the local microenvironment in the ectopic model does not support bone formation, further evaluation using an orthotopic model is needed.

Keywords: Multipotent (mesenchymal) stem cells, Differentiation



Surface modification of 3-dimensional synthetic polymer scaffold using hyaluronic acid: the effect on bone marrow stromal cells

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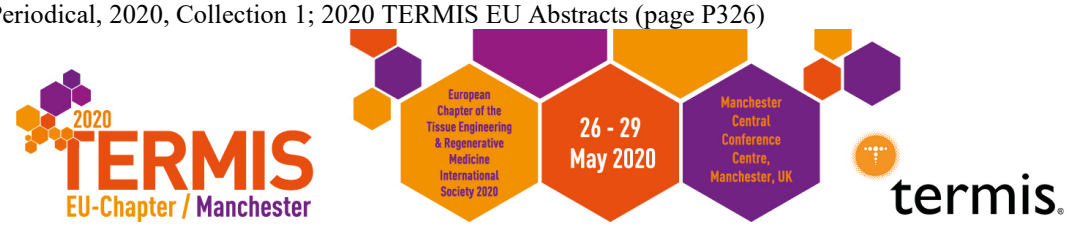
INTRODUCTION:Biomaterials used in tissue engineering applications should be biocompatible and degradable. Synthetic polymers can be tuned regarding mechanical strength, but are inherently hydrophobic. Functionalization by surface modification aims to increase cytocompatibility while maintaining the favorable properties of the biomaterial. Hyaluronic acid, a natural polymer, has not only excellent hydrophilic properties, but have also been shown to be an important part of the extracellular matrix and affecting physiological events such as wound healing.
Objectives
We hypothesized that surface coating of poly(L-Lactide co trimethylene carbonate) (PLLAcoTMC) scaffolds would enhance cell-biomaterial interactions.

METHODS:3-dimensional PLLAcoTMC scaffolds were coated with HA at concentrations 0.1%, 0.25% and 0.5% and unfunctionalized scaffolds served as control. Scaffolds were characterized using µCT. Human bone marrow stromal cells (BMSCs) were seeded on the scaffolds and cultured for up to 21 days. Cell viability, proliferation and differentiation were evaluated. HA release from the scaffolds was measured using a turbidity assay.

RESULTS:HA coating did not significantly affect the physical properties of the scaffolds. The cell suspensions were more evenly distributed on the scaffold surface in the 0.5% HA coating group. After 3 days fluorescent staining showed superior cell distribution within the pore interconnectivity in the scaffolds coated with 0.5% HA compared to the other groups. However, multi-level linear regression demonstrated comparable viability and differentiation for all groups ($p > 0.05$). HA released from the scaffolds could no longer be detected after 5 days in culture.

DISCUSSION & CONCLUSIONS:Cell-biomaterial interactions were enhanced immediately after cell seeding onto the constructs. The coated scaffolds show potential as a cell-carrier in bone tissue engineering applications.

Keywords: Biomaterials, Stem cells – general



Telomerase activity regulation and differentiation onset are oxygen-sensitive in differentiating pluripotent stem cells

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INTRODUCTION:Telomeres are guanine-rich repeats found at the ends of human chromosomes that protect chromosomes from degradation. Telomerase is a enzyme responsible for telomere elongation and maintenance where the telomerase reverse transcriptase gene (hTERT) encodes the rate-limiting catalytic subunit. Promoter methylation has been suggested to be associated with epigenetic control of telomerase regulation which holds important prospects for understanding cancer and stem cell biology. Control of downregulation of telomerase during differentiation of pluripotent stem cells provides a good model to study this endogenous regulation. Our previous studies showed that reduced oxygen culture conditions decrease global and DNMT3B methylation levels in undifferentiated and differentiated pluripotent stem cells. Here we sought to understand if reduced oxygen impacted on telomerase regulation during pluripotent stem cell differentiation.

METHODS:Pluripotent stem cells (SHEF1) were cultured in air oxygen standard incubator, 2%O2 controlled-oxygen workstation (2%WKS) and 2%O2-Pre-gassed media in a 2%O2 incubator (2%PG). E8 media (Life Technologies) and vitronectin-coated plates were used for SHEF1. Cells were spontaneously monolayer differentiated in Knockout DMEM, 10% FBS, 1% NEAA, 1% L-glutamine and β mercaptoethanol. Differentiated (day 5, 10, 20 and 40) and pluripotent cells were characterised with the human pluripotent stem cell markers (R&Dsystems, UK). Telomerase activity was measured with TRAPeze®RT Telomerase Detection Kit (Millipore, USA). Telomere length measured with Telomere Length Quantification qPCR Assay (Sciencell, USA). TERT gene expression and tri-germ layer differentiation markers were analysed using QPCR.

RESULTS:Pluripotency marker gene expression was consistent across all conditions in undifferentiated SHEF1 and gradually decreased during 20 days of differentiation, irrespective of oxygen condition. hTERT gene expression and telomere length significantly decreased in all conditions during 40 days of differentiation but surprisingly telomerase activity was significantly higher in reduced oxygen vs. air cultured cells during 40 days of monolayer differentiation. Further, in contrast to undifferentiated cells population doubling time was faster in hypoxia conditions for differentiated populations. Consistent with the above onset of tri-germ differentiation expression markers were significantly reduced in hypoxic oxygen vs. air cultured SHEF1 during the first 20 days of differentiation indicating an oxygen-linked rate-limiting step to differentiation.

DISCUSSION & CONCLUSIONS:In conclusion, telomerase activity was elevated and differentiation onset delayed in reduced oxygen cultured pluripotent stem cells. This suggests that telomerase activity is regulated in an hTERT transcript-level independent manner in physiological oxygen. Further studies are required to determine if promoter methylation and telomerase activity rates are linked.

Keywords: Differentiation

Scaled-up production of extracellular vesicles from an immortalised mesenchymal stromal cell line using xeno-free defined conditions

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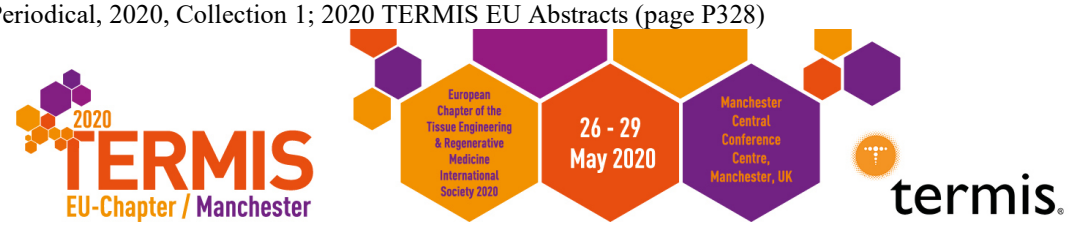
INTRODUCTION:Extracellular vesicles (EVs), which include microvesicles and exosomes, mediate intercellular communication by delivering proteins and nucleic acids as membrane-bound cargoes. The use of EVs as therapeutics has been widely explored in recent years, particularly in regenerative applications. One of the main challenges to the clinical use of EVs is scale-up, which demands a consistent cell supply grown in chemically defined, serum-free conditions to control product quality and eradicate contaminating EVs, growth factors, cytokines and batch-to-batch variability. Here we present a minimal, chemically defined medium (CDM) specifically designed for the expansion of an in-house engineered immortalised clonal human mesenchymal stromal cell (MSC) line and EV scale-up.

METHODS:We used a highly efficient Design of Experiments (DoE) approach to address the complex optimisation challenge of serum replacement. From a panel of candidate factors, we ultimately identified five additives, including mixtures of trace elements, lipids, and proteins, which were used in different combinations and concentrations in a basal culture medium to produce the CDM. Screens were made using an immortalised human MSC line (Y201). CDM performance was measure by assessment of MSC morphology, growth and viability using Realtime Glo, PicoGreen and Alamar Blue assays. Scale-up was achieved using a continuous perfusion hollow-fibre bioreactor (Quantum, TerumoBCT, 1.7m2 surface area). EVs were isolated from the spent medium collection bag (on ice over the last 24h of cell expansion) by ultracentrifugation to produce a 10,000xg “microvesicle” fraction and 100,000xg “exosome” fraction.

RESULTS:The CDM was able to support the adhesion, growth and passaging of Y201 MSCs using standard tissue culture plastic and maintain MSC viability at levels equivalent to cells grown in 10% serum-containing medium and significantly above no serum controls ($p<0.05$). CDM supported EV production by Y201 MSCs collected from the Quantum bioreactor, which were identified by transmission electron microscopy and western blot detection of flotillin and HSP70. Using nanoparticle tracking analysis we observed a 3-fold increase in exosome production (1.32 x 1010 versus 4.52 x 109 particles/million cells) and more varied size ranges (major size peaks at 27nm, 65nm, 104nm, and 139nm versus 115nm and 151nm) in the Quantum bioreactor compared to standard static culture conditions, whereas microvesicle yields and sizes were broadly similar.

DISCUSSION & CONCLUSIONS:The combined use of a reproducible, immortalised MSC line with xeno-free defined culture conditions enables the continuous scaled-up preparation of significant numbers of EVs suitable for clinical application.

Keywords: Extracellular vesicles, Biologics and growth factors



The role of endothelial progenitor cells with myeloid characteristics in a subcutaneous in vivo vascularization model

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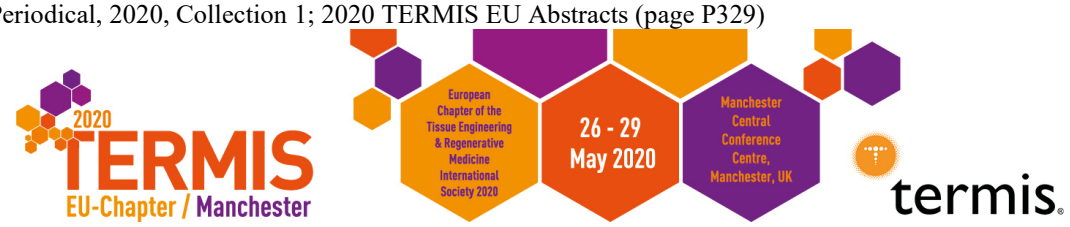
INTRODUCTION:Human peripheral blood derived endothelial progenitor cells are discussed to contribute to the formation of new blood vessels either by differentiation into outgrowth endothelial cells (OEC) or by functioning as accessory cells promoting the vascularization process via paracrine factors and diverse differentiation capacities. Nevertheless, the role of undifferentiated early endothelial progenitor cells (EPC) is still elusive regarding their impact on the vascularization process and their differentiation in vivo. We have previously shown that EPC enhance the formation of vascular structures in in vitro angiogenesis models based on triple cultures with OEC and bone mesenchymal stem cells. In this study we injected EPC in different combinations with OEC or MSC subcutaneously into a mouse model, quantified the formation of human blood vessels and evaluated the phenotype and localization of EPC via staining for TRAP (Tartrate-Resistant Acid phosphatase) and CD163 as a marker for M2 macrophages.

METHODS:In 6 independent sets of experiments different cell combinations 1) MSC (100%) 2.) MSC + OEC (50/50%), 3. MSC + OEC + EPC (50,50,10% 4.) MSC + EPC (100, 10%) were injected subcutaneously into the neck of beige scid mice (n=3) using Matrigel©. After 2 weeks animals were sacrificed and Matrigelplugs were removed. These samples were subjected to histology for human specific CD31 to monitor the vascularization process and co-stained with CD163 to monitor M2 macrophage development. In addition, TRAP staining was applied for macrophage or preosteoclast cell phenotypes identification. Hematoxylin was used for nuclear counterstaining. Sections were evaluated using EVOS Imaging system and Celleste Software for quantitative evaluation. Statistics were performed based on mean and SD values for all donors but also for the individual cell donor combinations.

RESULTS:In all experiments human cell derived blood vessels were observed in groups which contained outgrowth endothelial cells. Morphologically these blood vessels indicated an active perfusion. In average including all donor sets, EPC did not improve the vascularization investigated after 14 days but this impact was highly donor specific. Nevertheless, TRAP and CD163 were approved as suitable markers to identify human EPC and indicated their macrophage or proangiogenic M2 phenotype as well as co-localization with vascular structures. Interestingly, the M2 macrophage marker CD163 was most prominent in the MSC /EPC group although the ratio of EPC was identical in the MSC/OEC/EPC group indicating an impact on EPC differentiation.

DISCUSSION & CONCLUSIONS:Early EPC function as M2 Macrophages, although their impact on vascularization in vivo needs assessment via longitudinal assessment methods.

Keywords: Bone and bone disorders (osteoporosis etc), Stem cells – general



Cell Sheet Engineering and 3D Culture Approaches to Develop Artificial Vasculature

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INTRODUCTION:The generation of stable vascular beds to supply large artificial tissues and explore vascular processes in health and disease remains a major challenge in tissue engineering. By means of cell sheet engineering and 3D angiogenesis assays we are developing multilayered vascular tubes as well as microvascular beds. Alone or in combination they can be powerful tools for the development of 3D vascularized in vitro models and research in vascular biology.

METHODS:Thermoresponsive poly(glycidyl) ether (PGE)-coated cell culture substrates were used to fabricate and harvest confluent cell sheets of endothelial cells, smooth muscle cells and fibroblasts by temperature reduction. Tools were developed to roll the cell sheets onto a temporary template. For sprouting assays, 3D cultures of human umbilical vein endothelial cells were established and analyzed by microscopy and ELISA.

RESULTS:Conditions for the growth and temperature-triggered detachment of cell sheets of the different vascular cell types [1] and their co-cultures from the PGE-coated surfaces were successfully determined. The cell sheets could be rolled up onto a temporary template into tubular structures. 3D microvascular networks with long-term stability up to 21 days were achieved.

DISCUSSION & CONCLUSIONS:Cell sheets of three vascular cell types can be detached temperature-triggered from our poly(glycidyl) ether (PGE) surfaces and rolled into tubular structures. The procedure will be further refined and the vascular structures will be cultured in a bioreactor under flow conditions for further tissue maturation. In combination with the established methods to induce vascular sprouting in a 3D culture, long-term stable and perfusable vascular beds can be generated.

ACKNOWLEDGEMENTS:We thank the Federal Ministry of Education and Research for financial support (FKZ 13N13523).
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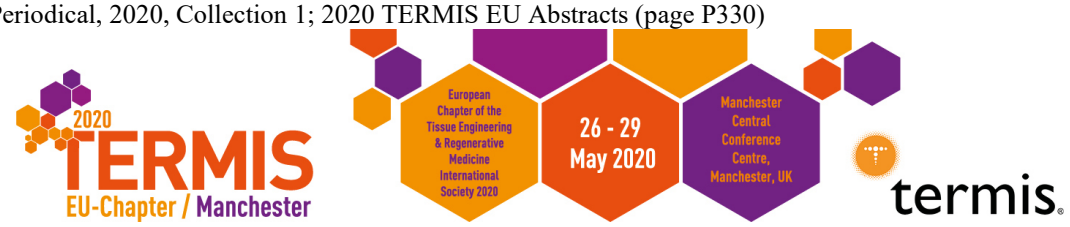
Keywords: Polymers - natural / synthetic / responsive, In vitro microenvironments



Flow and Form: Imaging biomechanical parameters to study the vascular tree formation within developing chicken embryo

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INTRODUCTION:To be clinically effective, engineered tissues should be mechanically stable with surgical compatibility and have a multiscale hierarchical organization resembling vascular tree. Apart from organization, hemodynamic parameters such as blood flow velocity, patency and vascular permeability are the important parameters which control the vascular structures to pass over remodeling and maturation phase [1]. However, how developing vascular network acquire hierarchical organization, what signals drive the organization, and what parameters are involved behind the scenes, remains poorly understood. This study reports the application of optical techniques, namely laser speckle contrast imaging (LSCI), laser Doppler perfusion imaging (LDPI) and monitoring (LDPM) and side-stream dark field imaging (SDF) [2] to explore and quantify the abovementioned hemodynamic parameters within developing chicken embryo.

METHODS:To follow how the vascular networks form around the yolk, fertilized chicken eggs were cracked on day 3 into a PDMS container. The imaging procedure of different techniques is listed below;
1. Non-invasive (LSCI and LDPI): The embryo surface is illuminated by a uniform coherent light distribution and imaged via cameras.
2. Invasive: (a) LDPM: Optical probe containing a pair of fibers to illuminate coherent light and detect Doppler shifted photons is mounted on a vessel.
(b) SDF: Optical probe including visible light emitting diodes (LEDs) and photodetector is mounted on the capillaries to image the dark field.

RESULTS:Both qualitative and quantitative assessment of spatiotemporal blood flow perfusion maps and RBCs velocity measurement were performed within macro- and microvascular structures. Results show that relative perfusion levels of individual blood vessels can be imaged with LSCI and LDPI in full-field with millimeter resolution. It can also be locally monitored in time domain using LDPM. Moreover, SDF helps to probe the formation of microcapillaries, and quantitatively measure the blood flow within the capillaries with micrometer resolution.

DISCUSSION & CONCLUSIONS:By developing an ex ovo chicken embryo system that is compatible with multiple imaging technologies, we have developed a powerful tool to better understand the processes of vascular organization and maturation. Next steps will focus on the perturbation of the vascular network using mechanical and chemical signals to study how these signals regulate the organization. This will provide crucial information to design mechanically stable organized vascular networks within engineered tissues.

Acknowledgements:This work is supported by an ERC Consolidator Grant (724469) and NWO Dutch funding (14538).

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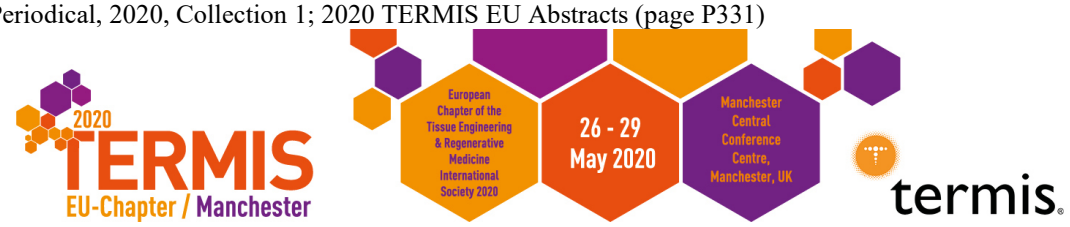
Keywords: Imaging - advanced, Biomechanics / biophysical stimuli and mechanotransduction



Heterotypic cell interactions regulate stromal cell overgrowth of primary microvascular endothelial cell cultures derived from human adipose tissue

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INTRODUCTION:Adipose tissue is an attractive source of endothelial cells (ECs) for vascular tissue engineering because it can be harvested autologously in large quantities with minimally invasive procedures[1]. However, stromal cell overgrowth of primary microvascular EC (MVEC) cultures remains a formidable challenge[2]. The underlying dynamics were investigated to improve the efficacy of expanding adipose-derived MVECs (AMVECs).

METHODS:CD45–CD31+ AMVECs were isolated using immunomagnetic beads. Purity was assessed by flow cytometry (FCM). Proliferation rates of AMVECs and residual adipose-derived stromal cells (ASCs) from the cell sorting procedure were characterized by population doubling time (PDT) assays. AMVECs and ASCs were seeded in defined proportions to recapitulate different efficacies of enrichment for the CD45–CD31+ immunophenotype, and their resultant proliferation rates in co-culture were assessed by FCM. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to identify potential mediators of observed growth inhibitions.

RESULTS:Primary AMVEC cultures were enriched for the CD45–CD31+ immunophenotype when compared with adipose tissue in 3/20 isolates (98.3 ± 1.0% vs. 2.9 ± 0.6%, respectively; $p < 0.05$); the remaining 85% of primary cultures were overgrown by fibroblast-like cells, determined to be CD45–CD31– ASCs by FCM. AMVECs exhibited a significantly longer PDT than ASCs (51.3 ± 9.1 hours vs. 23.6 ± 3.3 hours, respectively; $p < 0.05$), and their proliferation was further suppressed with an increasing proportion of ASCs in co-culture ($p < 0.05$). Interestingly, AMVECs also inhibited the proliferation of ASCs when comprising an increasing proportion in co-culture, albeit to a lesser extent than the ASC-mediated AMVEC suppression ($p < 0.05$). Proliferation rates of AMVECs and ASCs in co-culture were significantly faster when separated by a semi-permeable membrane ($p < 0.05$), indicating that the growth inhibition was at least partially contact-dependent. LC-MS/MS detected 924 differentially expressed proteins between AMVECs and ASCs (FDR < 0.05), and an interaction analysis of those localized to their plasma membranes identified 56 that may be implicated in this heterotypic cell contact-dependent growth inhibition and are the subject of ongoing investigations.

DISCUSSION & CONCLUSIONS:Prevention of stromal cell overgrowth of primary AMVEC cultures requires that the AMVEC-mediated growth inhibition of residual ASCs from the cell sorting procedure exceed the more potent ASC-mediated suppression of the inherently slower proliferation rate of AMVECs. The mechanisms underlying this heterotypic cell contact-dependent growth inhibition are the subject of ongoing investigations, and may be exploited to facilitate the targeted growth inhibition of residual ASCs in primary AMVEC cultures.

Acknowledgements:CIHR#230762

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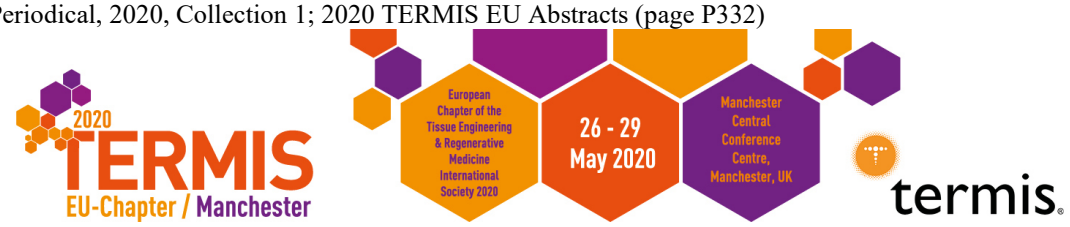
Keywords: Cardiovascular, Cell therapy



VEGF-decorated fibrin matrix enables rapid and physiological self-assembly of micro-vascular networks in vitro

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INTRODUCTION:In vitro engineered 3D tissues are a very promising area for a variety of applications, including drug screening and regeneration. However, it is still challenging to generate a physiologically structured vascular component, although it plays key functions in the tissues. The formation of vascular networks in vivo crucially relies on highly controlled signaling from morphogenic factors like Vascular Endothelial Growth Factor (VEGF). In particular, VEGF requires interaction with the extracellular matrix to provide physiological signaling. Here we took advantage of protein engineering technology to decorate fibrin hydrogels with recombinant VEGF and rapidly and robustly generate self-assembling microvascular networks in vitro.

METHODS:Fibrin matrices were prepared by mixing 2.5 or 10 mg/ml of human fibrinogen, 0.2 or 3 U/ml of thrombin and 3 U/ml of factor XIIIa combined with Human umbilical vein endothelial cells (HUVEC) and adipose derived stromal cell (ASC) alone or in combination (1:1 ratio) at a total density of $1x10^6$, $5x10^6$ or $10x10^6$ cell/ml. VEGF164 was fused to the transglutaminase substrate octapeptide NQEQVSPL (TG-VEGF), to allow its covalent cross-linking into fibrin hydrogels (final concentration 10 and 100 ng/ml). Hydrogels were immunostained for laminin and podocalyxin and vessel length density (VLD) was quantified.

RESULTS:A fibrinogen concentration of 10 mg/ml was required to ensure a true 3D cell distribution throughout the hydrogel volume. Co-culture of HUVEC and ASC, but not either alone, gave rise to a physiologically differentiated vascular network with clear apico-basal polarization of endothelium, marked by basal laminin and luminal podocalyxin. Moreover, vessels displayed well-formed open lumens about 20 µm in size, in the range of physiological micro-vessels. The efficiency of vessel formation was increased by both cell density and the addition of TG-VEGF. TG-VEGF also accelerated the kinetics of vascular network assembly. Use of $5x10^6$ cell/ml with 0.1 µg/ml of TG-VEGF yielded the maximum efficiency and speed of network formation, with structures already forming after 4 days

.

DISCUSSION & CONCLUSIONS:We optimized conditions to rapidly generate a self-assembled functional microvascular network in vitro. Fibrin gel composition, cell density and the provision of a specific morphogenic cue (TG-VEGF) have been identified as key parameters that determine the number of vessels and the kinetics of their formation.

Acknowledgements:This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 801159.

Keywords: Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models

Electrospun produced 3D covering for vascular stents: ex vivo and in vivo study

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INTRODUCTION:Restenosis after artery stenting is an urgent problem of transluminal angioplasty. Covering of vascular stents (VS) with drug-eluted matrices produced by electrospinning (ES) can solve a problem mechanically support damaged tissues and inhibiting cell proliferation. Here we explore penetration of paclitaxel (PTX) from previously described ES matrices [1] through arterial wall ex vivo and their effects on stenting efficacy in vivo.

METHODS:Coating of 130–150 μm with ~0.46 μg/cm2 PTX (~26000 cpm/cm2 of tritium labeled PTX was introduced for ex vivo experiments) was applied over metal VS (covered VS (CVS)) by ES [1]. CVS were placed onto balloon catheter and sterilized. To follow PTX diffusion through artery wall, CVS was installed in fresh rabbit iliac artery (RIA). Balloon with CVS (with and without RIA) were installed in 5 ml tube between two opposite holes and fixed by indeflator at 4 atm. PBS was introduced over CVS and PTX release was measured by radioactivity of PBS. VS and CVS were installed in rabbit iliac artery through carotid artery access and common trans-catheter delivery technique. Iliac artery patency was controlled by ultrasound Vividi system. After explantation, stented arteries were studied by survey microscopy (Stereo Discovery V12, Zeiss); histological study was executed after fixation of stented arteries in acrylate resin [2].

RESULTS:Arterial wall retards PTX release up to 3-4 times during first hour and to 2-2.5 times after 24 hours. After 24 hours of incubation with an arterial wall, more than half of the PTX released is retained in artery wall. Covering does not prevent stent delivery but prevent struts deepening into artery wall and its thickening. ES covering prevent decreasing of stented area effective crossection: after 6 month linear blood flow velocity (LBV) in VS group increase up to 2 times whereas in CVS only by 30% with tendency to stabilization after 3 month. Histological study confirm LBV data: extensive neointima was found in VS stented arteries, whereas narrow layer of neointima with well-formed parallel collagen fibers covered with single cell layer was observed in CVS.

DISCUSSION & CONCLUSIONS:Arterial wall retain PTX, capacity and retardation time are important for rational drug delivery design. ES produced covering significantly improves patency of stented arteries as shown by LBV and histologically.

Acknowledgements:The study was supported by RSF project 18-15-00080.

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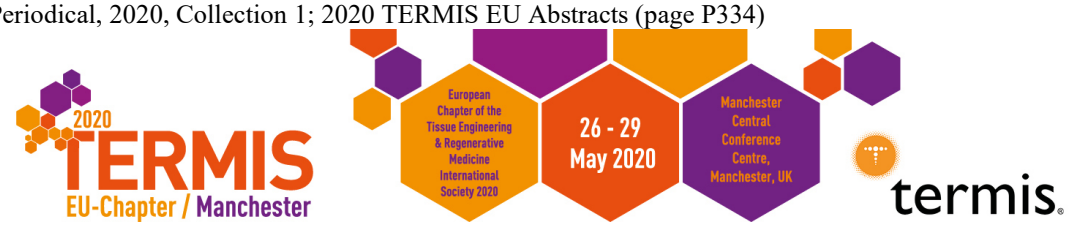
Keywords: Drug delivery, Vascular systems / vascularisation and heart



Endothelial Cell Network Formation In Coculture Setting under Defined Culture Conditions

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INTRODUCTION:Non-vascularized tissue-engineered constructs are restricted in size due to the limitation for nutritional supply of the integrated cells by diffusion. Therefore, as a minimal requirement a vascularization needs to be implemented into 3D tissue engineered constructs. In this study a self-assembly strategy of endothelial cells in co-culture with mural cells was employed addressing: a) hydrogel generation from xeno-free components, b) culture of construct under defined serum-free conditions and c) the characterization of the generated construct.

METHODS:GFP labeled human umbilical vein endothelial cells (EC) were combined with human adipose-tissue derived stromal cells in a hydrogel cast onto decellularized small intestine submucosa and cultured in either endothelial cell growth medium-2 or serum free medium[1–2]. The hydrogel contained either Matrigel/rat tail collagen I or human collagen I derived from fibroblasts[2]. The generation of stable EC networks is observed under all conditions[2].

RESULTS:The 3D quantification of the network revealed a slightly thicker construct for the matrigel/rat collagen I-based hydrogel compared to human collagen I-based constructs[2]. Additionally, the ECs within the formed network were able to produce their own extracellular matrix proteins (e.g. Collagen IV). A staining against -smooth muscle actin (α-SMA) revealed the presence of α-SMA positive cells along the EC cords which seem to be in physical contact with EC[2]. Moreover, Texas red-labeled dextran was detected in structures encircled by EC indicating the presence of hollow structures within the EC network[2].

DISCUSSION & CONCLUSIONS:Stable EC networks are generated in a xeno-free hydrogel in serum-free medium. The retrieved EC structures are hollow and enwrapped with α-SMA positive cells indicative of a microvascular EC network stabilized by pericytes[2]. This achievement represents a fundamental step towards a clinical applicable vascularized construct.

Acknowledgements:This work was financed by the CORTISS foundation, the Deutsche Forschungsgemeinschaft (Project HA 13 06/9-1), the BMBF Project “AUREKA” and DFG Cluster of Excellence “REBIRTH”

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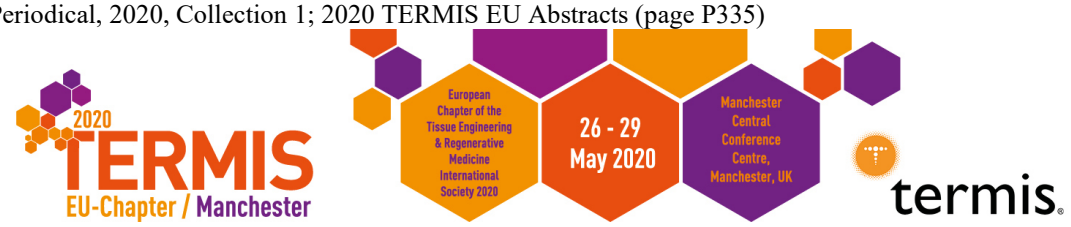
Keywords: Decellularised matrices, Cardiovascular



Development of a bilayer blood vessel substitute for vascular tissue engineering: an in vitro study

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INTRODUCTION:Cardiovascular diseases may be associated with atherosclerosis or damaged arteries of organs. Tissue engineering is a promising approach to overcome limitations of autografts or synthetic grafts, like intimal hyperplasia and infection. The challenge in vascular tissue engineering is to imitate the layers of native arterial wall. Aim of this study is to develop a bilayer, small-diameter arterial substitute to mimic arterial intima and media layers, and to evaluate the construct under in vitro conditions.

METHODS:Tubular bilayer scaffold was composed of inner porous film and outer fibrous mat. The porous film was prepared by dip coating using a blend of poly(caprolactone) and poly(D,L-lactide-co-glycolide). Polyethylene glycol was also added as a porogen into the polymer solution. Aligned fibers were fabricated over the tubular film by electrospinning using a blend of poly(caprolactone), poly(D,L-lactide-co-glycolide) and poly(L-lactide-co-D,L-lactide). The inner side of tube was seeded with endothelial cells (HUVEC), while the outer fibrous mat was seeded with Wharton's Jelly mesenchymal stem cells (WJ MSC), which will be differentiated into smooth muscle cells in further studies. Cell proliferation on the scaffold was determined with WST1. The cell distribution and orientation on the scaffold were investigated by confocal microscopy after FITC-Phallodin and DAPI staining.

RESULTS:The vascular scaffold with a lumen diameter of ~3 mm was obtained successfully. Scanning electron microscopy results revealed that the thickness of the tubular film part and the outer fibrous layer was 20 µm and 35-40 µm, respectively. The tubular film layer exhibited porosity with an average 2-7 µm pore size. The circumferentially aligned electrospun fibers with a diameter of 1-1.5 µm were collected successfully over porous tubular film. It was observed that HUVEC cells attached and evenly distributed on the inner side of the tubular film. Confocal microscopy results demonstrated that WJ MSC responded to the anisotropfic topography of the outer fibrous mat and aligned along the axis of the fiber. It was observed that WJ MSC were organized circumferentially in the outer layer as planned to mimic the media layer of blood vessels. HUVEC and WJ MSC proliferated on the inner film side and on the outer fibrous mat, respectively.

DISCUSSION & CONCLUSIONS:The developed bilayer blood vessel substitute involving inner endothelial cells and outer circumferentially aligned WJ MSCs layers would be a potential therapeutic approach in regenerative medicine for artery diseases.

Acknowledgements:This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) SBAG 118S587 and TUBITAK 2210-C BIDEB Fellowship.

Keywords: Biomaterials, Multipotent (mesenchymal) stem cells

Flow-driven vascular network organization in a chorioallantoic membrane model

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INTRODUCTION:Adding vascular network to engineered tissues is an important step for the clinical application of these tissues. Recent studies have shown that vascular cells can display plasticity with respect to local cues, meaning that the organization is influenced by the environment and not just by genetics [1, 2]. Since it is clear that vascular cells are programmed to respond to hydrodynamic signals, and by considering the challenging nature of internal fluid flow control within vessels, we hypothesize that fluid flow shear stress on the outside of a vascular network can also guide vascular organization. As a natural highly vascularized model, the chick embryo and its chorioallantoic membrane (CAM) is applied in this project.

METHODS:An artificial cubic eggshell is designed in a way that allows oxygen passage into the chick embryo as well as high observability. A patterned membrane fabricated by microfabrication techniques is used in one of the walls of the artificial cubic eggshell to exert external fluid shear stress on CAM. The shear stress profile in the designed pattern is modeled using COMSOL Multiphysics software tool. The spreading vascular network is visualized using optical color imaging and the degree of vasculature revealed by the vessels-occupied area are calculated by using ImageJ software.

RESULTS:The results of this project show how external fluid flow shear stress affects vascular development and organization. When the hypothesis holds true, the results of this project will provide us with an extra tool to control vascular organization in engineered tissues.

DISCUSSION & CONCLUSIONS:If the external fluid flow shear stresses could control vascular organization, the blood vessels can be designed and reorganized without disturbing blood flow within the vascular network. This method can open new horizons in developing a functional vascular network within engineered tissues, which is a key challenge in tissue engineering.

Acknowledgements:This work is supported by an ERC Consolidator Grant under grant agreement no 724469. NSN is supported by a NWO Grant under grant agreement no OCENW.XS.021.

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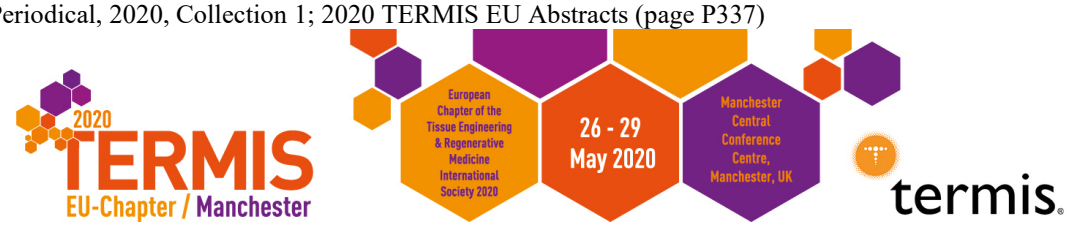
Keywords: Biomechanics / biophysical stimuli and mechanotransduction, Organ-on-a-chip / lab-on-a-chip / organoids and ex vivo models



Sustained DMOG delivery increases VEGF release of adipogenic stem cells for vascularization of tissue engineered implants

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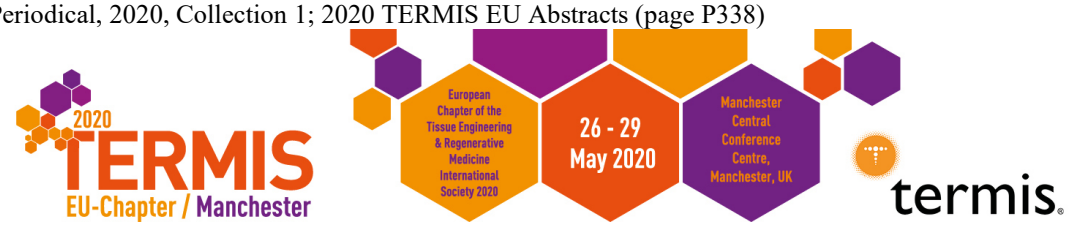
INTRODUCTION:The overall aim of the project is the development of tissue engineered implants, which are pre-vascularized in vitro, in order to enable better and faster implant integration and survival in vivo. In previous studies it has been shown that the induction of hypoxia stimulates angiogenesis in many tissues. Dimethyloxalglycine (DMOG), which is a prolyl hydroxylase inhibitor, can be used to chemically induce hypoxia, which stimulates adipogenic stem cells (ASC) to increase the production of VEGF, the key mediator in angiogenesis. However, previous experiments in this study have shown that a single dose of DMOG to ASC does not result in sustained VEGF production of the cells and repeated doses are needed. As fresh DMOG delivery to the implant would not be possible, porous silica nanoparticles (siNP) were used as delivery system for a sustained release of DMOG in the implant. This work addresses the question, whether VEGF production of ASC and cell viability can be sustained by a single dose of DMOG loaded siNP, in contrast to repeated doses of fresh DMOG or DMOG loaded siNP.

METHODS:ASC received either one or three doses of 0, 100 or 500 µM, either loaded on siNP or dissolved in media, over a period of nine days with three time points. Cell viability was tested using WST8 assay to quantify cell recovery as compared to the control group treated with 0 µM DMOG. VEGF ELISA was performed to quantify VEGF production of ASC normalized to the cell's viability.

RESULTS:ASC treated with repeated doses of DMOG dissolved in media showed sustained secretion of VEGF over nine days, whereas VEGF secretion of cells treated with a single dose of dissolved DMOG did not recover after an initial increase. When administering siNP loaded with DMOG in a single dose, VEGF production was increased and maintained at 5-fold higher on day nine, with the maximal secreted VEGF concentration of 15 ng/ml, compared to the basal VEGF concentration of 3 ng/ml of the control, whereas repeated administration of siNP loaded with DMOG resulted in an initial increase in VEGF production on day three, which was followed by a significant decrease ($p<0,0001$) on day six.

DISCUSSION & CONCLUSIONS:Single dosage of DMOG on siNP resulted in sustained VEGF secretion of ASC. Therefore, siNPs are a valuable tool for sustained release of VEGF, while maintaining cell viability and are suitable for prevascularization of implants, which will be tested in further experiments.

Keywords: Drug delivery, Hydrogels and injectable systems



Promotion of Pro-Angiogenic Secretome from Mesenchymal Stromal Cells Attached to Hierarchically Structured Biodegradable Microcarriers

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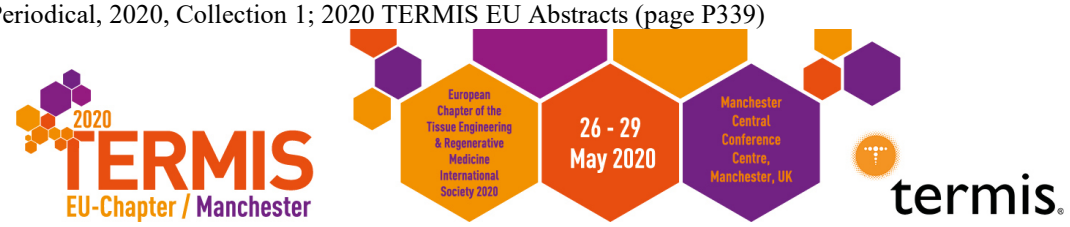
INTRODUCTION:Adipose-derived mesenchymal stromal cells (AdMSC) release multiple pro-angiogenic factors capable of stimulating neovascularization. Improved methods for delivery of their pro-angiogenic potency are sought. Substrate surface topography is a parameter known to manipulate the behaviour of AdMSC but challenges exist with translating this parameter into implantable materials. The aim of this study was to investigate three compositions of hierarchically structured, highly porous biodegradable implantable microcarriers for the culture of AdMSC and the influence of their surface topographies on the angiogenic secretome.

METHODS:Hierarchically structured microcarriers composed of 3 compositions of poly(DL-lactide-co-glycolide) that differ in the ratio of lactide:glycolide (75:25, 50:50 and 85:15) were prepared using thermally induced phase separation. Solid PLGA microcarriers were prepared using an oil-in-water emulsion solvent-evaporation technique. The physical attributes of the different compositions of microcarriers were characterized using scanning electron microscopy, nano computed tomography and porosimetry. Human AdMSC were cultured on the surface of the microcarriers for up to 11 days and supernatants collected at defined time intervals. Cell viability was examined via Live-Dead staining and measurement of lactate dehydrogenase. The secretome in supernatants was analysed using a Proteome Profiler Angiogenesis Array and ELISA and the pro-angiogenic activity evaluated using an in vitro model of angiogenesis.

RESULTS:All three polymer compositions produced hierarchically structured microspheres that performed well as cell microcarriers, facilitating AdMSC attachment and retaining their capacity for tri-lineage differentiation. The secretome in supernatants collected from AdMSC attached to the textured microcarriers contained multiple pro-angiogenic factors, with the levels of several pro-angiogenic factors including basic fibroblast growth factor, hepatocyte growth factor, platelet derived growth factor, placental growth factor-1, IL-1β and vascular endothelial growth factor (VEGF) increased compared with AdMSC cultured on control microcarriers composed of solid polymer or polystyrene. Quantification of VEGF in the supernatants collected from AdMSC attached to the textured microcarriers revealed significantly elevated levels compared with supernatants collected from cells cultured on tissue culture plastic at all time-points. Supernatants collected from microcarriers composed of 75:25 PLGA stimulated an increase in the number of tubules and junctions compared with control wells.

DISCUSSION & CONCLUSIONS:The unique properties of hierarchically structured, highly porous biodegradable microcarriers investigated in this study offer a radically different and transformative approach for achieving targeted delivery of the pro-angiogenic secretome from AdMSC that could be used to induce neovascularization in ischaemic tissue.

Keywords: Advanced therapy medicinal products, Cardiovascular



Hierarchically Structured Porous Biodegradable Films Promote Therapeutic Angiogenesis

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INTRODUCTION:Cardiovascular disease requires treatments to improve blood flow to ischaemic tissue. Therapeutic angiogenesis involving delivery of angiogenic growth factors has not translated well into clinical efficacy. A materials-based approach would provide a paradigm shift in treatment options available. Biophysical cues such as stiffness and material topography are important regulators of cell behaviour and influence secretion of growth factors. These features could be used as stimuli to trigger the endogenous release of angiogenic factors. The aim of this study was to investigate a materials-based approach that stimulates angiogenesis and increased tissue perfusion.

METHODS:Hierarchically structured porous biodegradable films composed of 75:25 Poly(D,L-lactide-co-glycolide) (PLGA) were prepared using thermally induced phase separation (TIPS). Solid control films were prepared by dip coating. SEM images of the polymer film surface were analysed using Image Pro software. X-ray micro computed tomography analysed the internal structure and atomic force microscopy was used to measure surface roughness. The mechanical properties of the polymer films undergoing simulated physiological degradation were characterized using indentation. The polymer films were overlaid onto the site of vascular occlusion in a murine hind limb ischaemia model. Blood perfusion was measured using laser Doppler imaging. Gene expression of angiogenic related growth factors in the treated tissue was measured using an RT2 Profiler PCR array. Blood vessel density was quantified in tissue sections immunostained for anti-Von Willebrand Factor.

RESULTS:TIPS polymer films exhibited an open porous surface with predominantly circular pores on the surface ranging in diameter from 0.5 µm – 8 µm. A hierarchical porous structure existed throughout the entire thickness of the film. Simulated physiological degradation resulted in loss of surface porosity and roughness which coincided with a reduction in elastic modulus of the material over 21 days. Reperfusion was significantly increased at day 14 and day 21 in hind limbs treated with TIPS polymer films compared with control groups. Tissue surrounding the TIPS polymer films contained an increase in blood vessel density and an elevated level of several pro-angiogenic genes compared with control samples.

DISCUSSION & CONCLUSIONS:Hierarchically-structured macroporous biodegradable films produced using thermally induced phase separation provide a localized biophysical stimulus that results in restoration of blood flow to ischaemic tissue. The mechanism of action appears to be through local upregulation pro-angiogenic genes and increase in blood vessel density. These findings show that acellular biodegradable materials can provide an innovative approach for therapeutic angiogenesis to enhance tissue reperfusion in vivo.

Keywords: Cardiovascular, Biomaterials

Aggregates of equine mesenchymal stem cells with gelatin hydrogel microspheres for regenerative therapy of tendon injury

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INTRODUCTION:The implantation of mesenchymal stem cells (MSCs) for equine superficial digital flexor tendon (SDFT) injury has gained prominence as a treatment option. Recent studies have revealed that MSC aggregates cultured with biodegradable gelatin hydrogel microspheres (GMS) augment the biological functions[1]. We hypothesized that the implantation of MSC aggregates with GMS would enhance the efficacy of cell therapy. In this in vivo study, we evaluated the residual period of the administered MSC aggregates with GMS and compared the effects of this therapy to those of monolayer-cultured MSCs using ultrasonography.

METHODS:Aggregates were produced by culturing 10000 MSCs with 500 GMS in 96-well low attachment plates. In experiment 1, MSCs were labeled using a fluorescent cell labeling kit. MSC aggregates with GMS or monolayer-cultured MSCs (total number of MSCs was $1x10^7$, respectively) were injected into each forelimb SDFT with surgically induced injuries for six horses. After about 10 days, we measured the fluorescence intensity of frozen sections obtained from each SDFT.
Similarly, for experiment 2, we injected MSC aggregates with GMS or monolayer-cultured MSCs into 6 horses with surgically induced SDFT injuries. Ultrasonography examinations were performed at the maximal injured area to evaluate the proportion of hypoechogenicity cross-sectional area, the strain ratio of sonoelastography, and the vascular score of power Doppler ultrasonography (0–4) at baseline and 1, 3, 5, 7, 9, 11, and 13 weeks after transplantation. $P < 0.05$ was considered to be statistically significant.

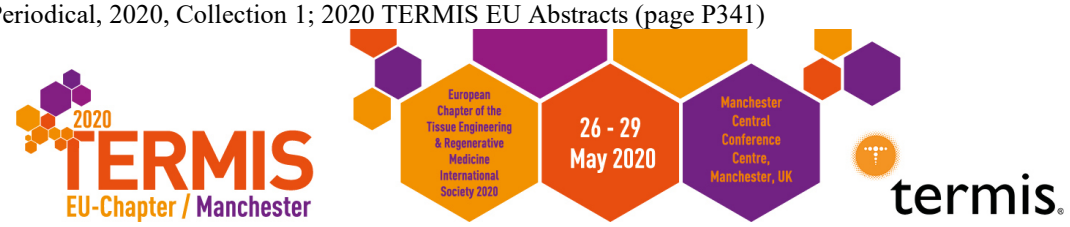
RESULTS:1. The fluorescence intensity of frozen sections obtained from aggregate-administered tendons was significantly higher than that of tendons treated with the monolayer-cultured cells ($P < 0.05$).
2. Compared to tendons treated with the monolayer-cultured cells, the aggregate-administered tendons had an early decrease in the proportion of hypoechogenicity cross-sectional area, an early increase in the strain ratio (both $P < 0.01$), and an earlier disappearance of neovascularization ($P < 0.05$) in the maximal injured area.

DISCUSSION & CONCLUSIONS:Our results suggested that the aggregates with GMS were effective for prolonging the residual period of MSCs in the equine injured tendons. The results of the ultrasonography findings suggested an early recovery of tissue stiffness and early termination of the inflammatory process in the lesion of the aggregates-administered tendons. Further research is required to establish a mechanism for the changes in repair of injured tendons, but aggregates with GMS appear to improve the efficacy of cell therapy.

Acknowledgements:We thank Keiko Kakuda for her cooperation.

References:1 Hayashi K, Tabata Y 2011 Acta Biomaterialia 7:2797-803

Keywords: Cell therapy



Oncolytic virus as immunotherapy treatment shows efficacy in canine cancer patients

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INTRODUCTION:The use of oncolytic viruses is an immunotherapy treatment for cancer based in the use of viruses designed to replicate specifically in tumor cells. This innovative approach has even received the approval from the FDA and EMA with Imlygic, an oncolytic virus based in herpex virus, to be used intratumorally in melanoma patients. Other clinical trials are supporting the increasing evidence about the use oncolytic adenoviruses (Ads) as promising immunotherapy agents. Our goal is determine the safety and efficacy of canine Ads to be used intratumorally. We perform a clinical trial treating canine patients with our Ad ICOCAV15.

METHODS:Canine cancer patients were diagnosed and included in our clinical trial. They were treated once intratumorally with 107 IU (500uL) of oncolytic adenovirus ICOCAV15 (distributed in 5 injections). We took two measures of the tumor weekly, and also we perform a computerized tomography the day of the treatment (d0) and 28 days after (d28). To evaluate the tolerance of ICOCAV15, peripheral blood was taken and analyzed at d0 and d28 to determine renal and hepatic damage, coagulation problems and hematopoietic disorders. 3 biopsies were taken at d0 and d28 to analyze the immune populations and also to evaluate the inflammatory status of the tumor. Tissues were cryopreserved with Tissue-teck compound, a dried piece frozen and a piece was embedded in paraffin.

RESULTS:In the 7 dogs treated to the date, we did not detect any secondary effects related to the treatment including the peripheral blood analysis. Results show that in two of the patients that curse with a squamous cell carcinoma the area of the external lesion decreases a 37.5% at d57 and a 65% at d21.

DISCUSSION & CONCLUSIONS:Treatment of canine tumor with ICOCAV15 intratumorally is safety, well tolerated and improve their life quality. In some cases, a clear clinical response is detected. In sum, oncolytic Ads would be a new tool to be used in veterinary oncology.

Keywords: Cancer, Veterinary

A marine-derived collagen-based skin substitute for skin regeneration: a preliminary study

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INTRODUCTION:Skin wounds are a challenging problem in the medical field and an economical burden. Skin substitutes (SS) are a promising alternative for skin wound healing since they mimic the natural skin structure and support its regeneration. Here, we present preliminary results about the application of an innovative marine-derived collagen-based SS in a secondary intention wound healing model.

METHODS:The collagen used to produce the SS was obtained from sea urchin (Paracentrotus lividus) food industry wastes, extracted in its fully native fibrillar and GAG-decorated form [1]. Two wounds (4x4 cm) were surgically induced on the back of three sheep: one of them was treated with the SS and one left untreated (placebo). Clinical evaluations for wound contraction and re-epithelialization were performed every week. At 7, 14, 21 and 42 days, biopsies were collected with a 6 mm² punch in order to perform histopathological (H&E) and molecular (qPCR) analyses. Histological analyses allowed to provide a score to the maturation of granulation tissue (GT) and skin adnexa. Gene expression analysis was performed for Collagen1, Collagen3, VEGF and hair-keratin (hKER).

RESULTS:Clinically, the SS led to a better re-epithelialization than the placebo while no differences in wound contraction were observed. Treated wounds showed a faster histological resolution of GT at 21 days, with no exuberant deposition of scarring tissue at 42 days; this result was further corroborated by the different expression levels of both collagen types at 14 and 21 days. Concomitantly, the higher expression of VEGF (day 14) was detected. In untreated wounds, on the contrary, GT appeared only at 14 days and did not mature properly leading to dermal fibrosis at 42 days. SS application led to the development of a larger amount and better developed skin adnexa (present since day 14) than untreated wounds: treated wounds started to express hKER since day 14.
DISCUSSION & CONCLUSIONS:At 42 days, a faster re-epithelialization, a better development of skin adnexa and a properly deposition of GT were observed in the SS-treated wounds while the untreated ones showed a hypertrophic epidermis and fibrosis, indicators of a tissue still in the process of healing. Interestingly, VEGF expression might have supported GT maturation. Therefore, these preliminary findings suggest an overall better skin repair with the SS treatment than the placebo. Future studies should compare these beneficial effects with a commercially available SS (e.g. Apligraf®).
References:[1] Ferrario et al. Mar Environ Res. Jul;128:46-57, 2017.

Keywords: Skin, Biomaterials

Mesenchymal stem cells insert to microcapsules – modulating a joint inflammatory response in horses

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INTRODUCTION:Horses are often affected by degenerative joint disease, resulting in low athletic performance. Therapy of mesenchymal stem cell derived from the synovial membrane has presented great therapeutic potential, but presents very rapid dispersion and decrease in viability when applied freely in the joint. The objective of this project was to evaluate in vivo the immunomodulatory potential of mesenchymal stem cells inserted in microcapsules, when administered intra-articular.

METHODS:Mesenchymal stem cells derived the synovial membrane of horses, the bank of the Laboratory of Regenerative Therapy Unesp Botucatu/SP, administered intra-articular in the free from and inserted in alginate hydrogel microcapsules, after condral lesion in the lateral troclea of talus. Proceeded with serial collection of synovial fluid at different times (0 hours; 24 hours; 48 hours; 96 hours; 144 hours; 14 days), for physical, chemical and cytological evaluation, in addition to the dosage of inflammatory mediators (serum amyloid A, IGF, substance P, IL-1∝, IL-6, INF-Ɣ, IL-10, TNF ∝, PGE 2).

RESULTS:The cytological analysis of the synovial fluid was similar in both groups, statistical difference in 0 hours with 24 hours ($P<0,05$). The total protein synovial fluid difference in the control group with the other groups ($P<0,05$) with 0 hours and 48 hours, remaining high in the group of free cells. In the statistical difference was observed in IGF, IL- 6 and TNF ∝, in relation to the free cells and control group, which on the other hand demonstrated a difference in the substance P and INF-Ɣ. In addition to the positive correlation in the free cell group between TNF ∝, INF-Ɣ and substance P, also correlating with the increased of the total protein.

DISCUSSION & CONCLUSIONS:The statistical difference observed in the initial moments explains the initial inflammatory, which may be due to the surgical procedure, since it was observed in all groups. Thus, together with the increase in total protein, we can infer that the mesenchymal stem cells modulated the inflammatory process, due to the return to the values of the initial moments, after their administration. In the group of free cells the correlation observed positively with the levels of TNF ∝, INF-Ɣ, substance P and with the number of total neutrophils of the synovial fluid, confirming the presence of the inflammatory process, and that it lasted longer, a fact not observed in the other groups, thus we can infer that administration of intra-articular free cells generated an increase inflammatory mediators.

ACKNOWLEDGEMENTS:FAPESP, CAPES

Keywords: Cell therapy, Cartilage / joint and arthritic conditions

Bacteriophages and Mesenchymal Stem Cells/Aggregates in/on Gelatine Microspheres for Infected Wound Healing

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INTRODUCTION:This study attempts to produce bifunctional gelatine microgels/spheres carrying bacteriophages as antibacterial agents and mesenchymal stem cells (MSCs) for regeneration of both soft and hard tissue as potential hybrid materials for combinational therapy.

METHODS:Both plain gelatine and its cationized forms were used to prepare spherical microgels by dispersing gelatine solution within an oil phase to obtain water-in-oil dispersions that resulted spherical beads which were then cross-linked de-hydrothermally. As a model target Escherichia coli and its specific bacteriophages were propagated in standard bacterial cultures. Bacteriophages were labelled by fluorescence dyes for following of both loading and release on/from the gelatine microgels. Nanosemulsions carrying different amounts of bacteriophages were loaded by incubation dry gels with nanoemulsions in proper volumes - sucking the liquid phase by the dry gels.
MSCs were isolated from the adipose tissue from mice, cultured and aggregates were formed with or without the microgels both plain and the ones carrying bacteriophages. The aggregate forms/sizes, cell densities and viabilities were followed in two week's 3D cultures.

RESULTS:Gels with different cross-linking densities swollen in the aqueous phase at different extents were produced in the size range of 50-100 micron. Bacteriophage loadings within the microgels were almost 100%. The release of bacteriophages (both rate and amount) were different depending on the initial loading, properties of the beads (including size, cross-linked density, type of gelatine used) and environmental conditions (including temperature, pH, existence of specific digesting enzymes).
Note that gelatine gels were used as scaffolds for the cells/aggregates. The aggregate of the MSCs were in spherical forms with high cell densities and viabilities which were depended on the microgel formulation and 3D culture conditions.

DISCUSSION & CONCLUSIONS:The gelatine microgels were swollen in the aqueous phase at different extents mainly depending on cross-linking densities. The ratio of bacteriophage nanoemulsion volume and the amount of dry gelatine beads were adjusted to reach very high phage loading densities. Phage loading densities, gelatine bead properties and environmental conditions changed the release behaviour as expected. Successful MSCs aggregation around the gelatine spherical beads allowed us to prepare bioactive hybrids for tissue regeneration. These biofunctional hybrid materials - gelatine microgel beads containing bacteriophages and MSCs aggregates could be used especially for healing of infected wounds (both soft and hard tissue) which is under-investigation in vivo models.

Acknowledgements:E. Piskin has been supported by the Turkish Academy of Sciences as an honorary member.

Keywords: Infection, Stem cells – general

Treatment of Porcine Full-Thickness Wounds by Transplantation of Biodegradable Microcarriers Carrying Autologous Fibroblasts and Keratinocytes

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INTRODUCTION:Split-thickness skin grafts represent the gold standard for treatment of extensive skin wounds, even though their use is associated with poor cosmetic and functional outcome. To mitigate this problem, the use of tissue engineered skin substitutes has been suggested, often comprised of a combination of cells and biomaterials. Cells used should be autologous, while the biomaterial should be biodegradable to allow for the naturally occurring tissue remodeling. Rapid cell expansion and subsequent transplantation is desirable, preferably without the use of enzymes. The present study aimed at investigating the effects of transplantation of autologous keratinocytes (KCs) and fibroblasts (FBs) seeded on microcarriers in a porcine full-thickness wound model.

METHODS:Cells were isolated from full-thickness skin biopsies taken from pigs (sus scrofa domesticus, N = 8), expanded using standard cell culture protocols, and seeded on Porcine Gelatin Microcarriers (PGMs). The resulting tissue constructs were transplanted to 120 surgical full-thickness wounds (2 cm diameter) and covered with occlusive dressings. Treatment groups included NaCl control, PGMs, as well as PGMs seeded with FBs, KCs or both). Wounds were excised after one, two, four or eight weeks before histological and immunohistochemical analysis. All animal procedures were approved by the Regional Ethical Review Board (protocol no. ID534).

RESULTS:Histological analysis and quantification of cells on PGMs revealed optimal cell density and viability between 24-96 hours after seeding. Following transplantation, histological analysis revealed incorporation of the PGMs into the granulation tissue, with complete degradation after two weeks. Viable proliferating cells on and surrounding the PGMs were visualized using antibodies against pancytokeratin and Ki-67. The first two weeks after transplantation, PGMs seeded with cells resulted in significantly thicker neoepidermis compared to PGMs and NaCl controls ($p < 0.05$). Four and eight weeks after transplantation, no significant differences could be observed.

DISCUSSION & CONCLUSIONS:In conclusion, the experiments performed illustrate the successful use of PGMs as a biodegradable cell carrier for transplantation of FBs and KCs to full-thickness wounds. Moreover, autologous cells on PGMs significantly stimulates the re-epithelisation process. In conclusion, the proposed method is a promising candidate for cell expansion and subsequent transplantation, enabling delivery of large numbers of cells without the use of enzymes. Future studies will focus on additional outcome parameters to evaluate long-term quality of healing following transplantation.

Acknowledgements:The project received financial support from the Kamprad Family Foundation and ALF-Region Östergötland. The authors thank A Starkenberg and K Briheim for excellent technical assistance.

Keywords: Skin

Electrospinning wound dressings containing reactive oxygen species for tissue regeneration and antimicrobial applications

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INTRODUCTION:Skin is a complex tissue with a key protective and regulatory role; however, disease and trauma can adversely affect function. Accelerating the healing process whilst minimising the risk of bacterial infection is a crucial clinical challenge.

Antimicrobial agents have a significant effect in preventing and healing infected wounds. Honey has been used historically for its antibacterial and wound healing properties. The antimicrobial performance is due to the production of reactive oxygen species (ROS), high osmolality of sugar, and low pH [1]. ROS has been demonstrated to promote wound healing by encouraging cellular repair processes and guiding regeneration [1,2]. This study presents a novel electrospun mesh produced using Surgihoney® (SH) and polycaprolactone (PCL) or polyvinyl alcohol (PVA) for use in tissue engineering and wound dressing applications.

METHODS:Electrospun meshes were produced using either PCL or PVA incorporating different concentrations of SH. Electrospun meshes were characterised for their morphological, chemical, and biological properties. Morphology was examined using scanning electron microscopy, the presence of honey derived sugars was determined using mass spectroscopy and FTIR, and production of H_2O_2 was investigated through spectrophotometry. Preliminary cytocompatibility was assessed using human dermal fibroblasts and the antibacterial properties were determined through the disk diffusion method.

RESULTS:The mesh morphology mimics the nanoscale features of the extracellular matrix of the skin. FTIR characterisation and mass spectroscopy demonstrated that the SH was successfully incorporated into the mesh. Glucose was present in the PCL/SH meshes, however, H_2O_2 was not produced. The PVA/SH meshes indicated the presence of glucose and production of H_2O_2. Preliminary biological assessment indicates that the composite electrospun meshes are cytocompatible with high cell viability and proliferation. The PVA/SH meshes inhibited bacterial growth for up to 24 h.

DISCUSSION & CONCLUSIONS:Electrospun PCL/SH and PVA/SH meshes were successfully fabricated with the PVA/SH meshes demonstrating antimicrobial efficacy through production of H_2O_2. Enzymatic activity was inhibited in the PCL meshes thus H_2O_2 production was prevented. The PVA/SH meshes show promise in the development of a tissue regenerative and antimicrobial wound dressing.

ACKNOWLEDGEMENTS:Funding provided by the Republic of Turkey Ministry of National Education. Research supported by Matoke Holdings.

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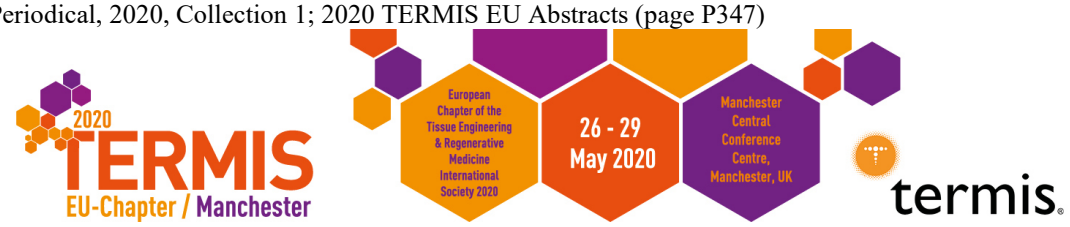
Keywords: Biofabrication, Skin



Cold Atmospheric Plasma Deposition to Initiate Wound Healing In Equines

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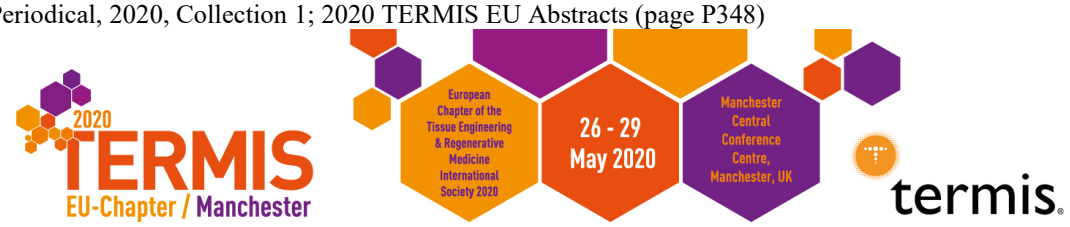
INTRODUCTION:Equines are, generally, excellent wound healers, as they are capable of repairing huge skin and muscle traumas. However, in some cases the healing process becomes compromised which can lead to the wound becoming infected. Our research is centered on using cold atmospheric plasma to improve the efficacy of wound healing in equines. The emergence of plasma medicine has provided some hope for advancement in wound closure rates for patients with chronic wounds and some positive clinical results have already been observed. However, the potential to combine antimicrobials and wound healing factors with plasma medicine has not yet been widely explored and this research examined one potential way to combine such therapies.

METHODS:A nebulised solution, containing collagen and/or antimicrobials, was introduced into the discharge of cold atmospheric plasma and the activated materials deposited onto a surface to produce an adherent coating. The plasma device was used to deliver the collagen and antimicrobials into bacterial and eukaryotic cultures and the antimicrobial, cytotoxic and wound healing effects were compared to untreated and plasma only treated controls.

RESULTS:Plasma deposited collagen promoted re-epithelialisation compared to the control. Although plasma treatment alone enhanced re-epithelialisation, the collagen treatment produced a statistically significant improvement in the rate of angiogenesis and re-epithelialisation. Plasma deposited antimicrobials showed an increased effect in bactericidal activity when compared with plasma treatment alone. The antimicrobials showed cytotoxic activity to eukaryotic cells at high concentrations, however, this was not seen at low concentrations.

DISCUSSION & CONCLUSIONS:High energy plasma devices have been shown to kill cells, cauterize flesh and fragment chemical precursors. Our data showed that treatment with plasma alone showed a beneficial effect for wound healing, while, higher levels of re-epithelialisation and angiogenesis were seen by the nebulised collagen. From this investigation, it can be deduced that exposing fibroblasts to cold atmospheric plasma can induce wound healing factors and this can be improved further by plasma deposited collagen. It has been known for a number of decades, that certain metal ions have antimicrobial activity, this has also been shown by cold atmospheric plasma. There has, however, been little research in the combination of plasma and other antimicrobials. Our investigations showed that plasma treatment alone had bactericidal effects and this effect was further improved by the deposition of antimicrobial metals via the plasma.

Keywords: Veterinary, Skin



Conductive hydrogels and wound healing: a multi-cue exploration

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INTRODUCTION:Combatting acute and chronic wounds is a large portion of healthcare budgets globally. Approaches to enhance and control wound healing often try to replicate individual stimuli that are present in vivo – for example: immune cell interactions, topography and biochemical manipulation. Cells communicate locally and distally via electrical signals and there is growing evidence of its importance in immunology. To understand wound healing and to create better in vitro models, we need to consider multicomponent and combinatorial approaches in applying physical, chemical and electrical cues. We are developing an in vitro model to explore these cues to better mimic the in vivo environment.

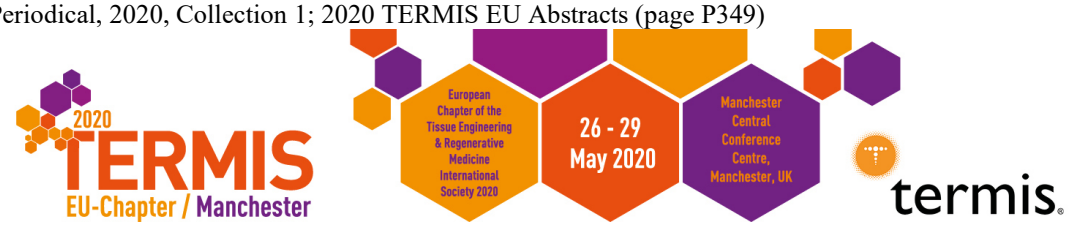
METHODS:Lung fibroblasts (MRC-5) and CD14+ monocytes derived from human blood – both integral in the wound healing process – were encapsulated in a three dimensional (3D) environment of small intestinal submucosal extracellular matrix (ECM) hydrogels (8 mg.mL-1). ECM hydrogels were prepared as 3D cell-encapsulated structures through manual pipetting methods and 3D extrusion printing, however prior to gelation they were modified to make them conductive with the addition of single and multi-walled carbon nanotubes (SWCNT and MWCNT, respectively) up to 0.2% v/v. Exogenous electrical stimuli was then applied using a voltage stimulator.

RESULTS:SEM showed structural differences when CNTs are added to the ECM. Mechanically, the MWCNT ECM hydrogels exhibited significantly higher storage moduli compared to the control and SWCNT ECM hydrogels ($P \leq 0.0002$); we think this is influenced by the visual structural changes as seen in the SEM images. Electrically, both types of CNT ECM hydrogels saw a significant decrease in impedance ($P < 0.0001$) and thus an increase in their conductivity compared to the control. Preliminary cellular studies show that the presence of ECM hydrogels, CNT embedded ECM hydrogels and electrical stimuli do not significantly affect the viability of MRC-5s or primary monocytes.

DISCUSSION & CONCLUSIONS:This is the first example of conductive 3D hydrogels combining the cell-compatibility of decellularised ECM and carbon nanotubes to study exogenous electrical stimuli. Future work will look to build on this data to show CNT ECM hydrogel structure and how they, along with the electrical stimuli, affect various cellular behaviours. The data presented and future work will go onto better replicate the complexity of the in vivo wound healing process compared to individual approaches.

ACKNOWLEDGEMENTS:Mr J. Jones Dr P. Sanjuan-Alberte.

Keywords: Decellularised matrices, Hydrogels and injectable systems



Antimicrobial nanofibrous materials from hydrophobized hyaluronan

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INTRODUCTION:The intended area of use of nanofibrous material is often wound healing, where it could serve as a barrier against bacteria and viruses not only for their porosity but also for the possibility to evenly incorporate various active ingredients (API, mostly antimicrobial). We prepared nanofibrous materials from hyaluronic acids (HA) usable as a dressing. HA plays an important role in processes such as tissue hydration, lubrication, or wound healing. HA is biocompatible, biodegradable and nontoxic. But native HA is highly hydrophilic, which makes materials from native HA unsuitable for longer use during medical treatment. To overcome this drawback, we focused on HA derivatives. Prepared nanofibrous materials are stable in a water-based environment and include antibacterial drugs – triclosan (TRI) and octenidine dihydrochloride (OCT).

METHODS:Nanofibrous layers were prepared via electrospinning of blends consisted of at least 90 wt.% of HA derivative, 2 – 5 wt.% of TRI or 0.5 – 2 wt.% of OCT and 2 – 9.5 wt.% of polyethylene oxide. A mix of isopropyl alcohol and water was used as a solvent. Prepared layers were analysed with SEM (fibre diameter) and HPLC (encapsulation efficiency). Also, the swelling behaviour of basic material and the antibacterial efficiency against five different types of bacteria were tested as well as drug release.

RESULTS:Two types of HA derivatives were used – hydrophobized (L-HA) and crosslinked after spinning (F-HA). The IPA/water soluble L-HA was used for water insoluble API encapsulation. F-HA was added to ensure porosity after swelling. The fibre diameter is not changed with a change in API content. HPLC shows good encapsulation efficiency for both types of API. Combining two types of HA derivatives we can maintain porosity of samples in PBS. Samples were stable after a week in PBS, no degradation was observed. A maximum of 20% of the total drug content was released in 72 hours from samples with TRI; the samples were active against C. albicans, E. coli, S. aureus and C. sporogenes. No release of OCT was observed after 72 hours, yet samples exhibited antibacterial activity against S. aureus.

DISCUSSION & CONCLUSIONS:By combining different types of HA derivatives we are able to prepare nanofibrous materials with maintainable stability in water-based solutions. The various content of antibacterial API could be evenly encapsulated and thus the properties of the targeted material (mechanical and biological) can be tuned for a specific application.

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Necas, et al. Veterinarni medicina, 2008

Keywords: Wound healing, Nanomaterials (inc graphene)

Skin substitute with artificial dermis and grafted keratinocyte sheets for full-thickness burn wounds

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INTRODUCTION:Although artificial dermis (AD) has been tested for wound bed preparation with cultured epidermal autografts (CEA), low CEA acceptance has been a challenging problem. Previously, we have reported effects of CEA overlaid on the wound bed grafted with ovine cadaver skin. The goal of the present study was to compare the efficacy of CEA overlaid on excised full-thickness burn wounds grafted with either AD or cadaver skin in sheep. We hypothesized that the effects of CEA overlaid on wound beds prepared with AD would be comparable to those prepared using cadaver skin.

METHODS:Epidermis of grafted CS started rejecting after 10 days, and its complete rejection was observed within 21 days. While, collagen sponge of AD was gradually changed to dermis-like tissue. The dermis thickness was comparable between CS and AD groups at 7 days (1907.5±122.4 vs 766.1±121.8 um, P=0.1), 14 days (2724.0±518.1 vs 2001.5±120.7 um, p=0.4), or 20 days (1814.6±93.0 vs 1724.0±121.3 um, p>0.9999). The percentage of wound epithelialized area after keratinocyte sheet grafting was also comparable between CS and AD groups at 7 days (50.1±7.8% vs 54.2±10.5%, p=0.76) or 14 days (91.2±4.1 vs 87.4±5.8%, p=0.60). The transmission electron microscopy analysis revealed no significant difference in the percentage of lamina densa between CS and AD groups (54.9±1.5 vs 52±1.3%, p=0.18). The number of hemidesmosomes per micrometer was similar between CS and AD groups (1.34±0.04 vs 1.3±0.06, p=0.62).

RESULTS:Epidermis of grafted CS started rejecting after 10 days, and its complete rejection was observed within 21 days. While, collagen sponge of AD was gradually changed to dermis-like tissue. The dermis thickness was comparable between CS and AD groups at 7 days (1907.5±122.4 vs 766.1±121.8 um, P=0.1), 14 days (2724.0±518.1 vs 2001.5±120.7 um, p=0.4), or 20 days (1814.6±93.0 vs 1724.0±121.3 um, p>0.9999). The percentage of wound epithelialized area after keratinocyte sheet grafting was also comparable between CS and AD groups at 7 days (50.1±7.8% vs 54.2±10.5%, p=0.76) or 14 days (91.2±4.1 vs 87.4±5.8%, p=0.60). The transmission electron microscopy analysis revealed no significant difference in the percentage of lamina densa between CS and AD groups (54.9±1.5 vs 52±1.3%, p=0.18). The number of hemidesmosomes per micrometer was similar between CS and AD groups (1.34±0.04 vs 1.3±0.06, p=0.62).

DISCUSSION & CONCLUSIONS:Our results suggest that use of AD can successfully substitute cadaver skin for preparing wound beds. Our results also demonstrate that AD and autologous keratinocyte sheets can substitute autologous skin grafts.

Acknowledgements:N/A
References:N/A

Keywords: Wound healing, Skin

Antibacterial lawsone loaded nanocellulose-chitosan cross linked porous scaffold accelerates cutaneous wound healing

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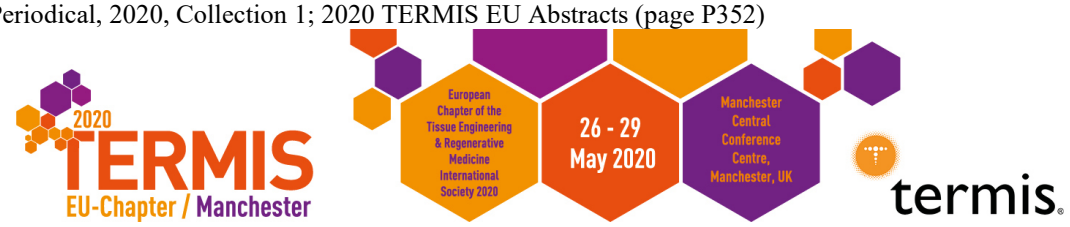
INTRODUCTION:Cutaneous wound resulted from severe accidents; burn; surgeries have been major global issues along with lowering patients' quality of life. Though skin has self-healing capabilities but process is lengthy. The healing become much slower due to microbial infections, delayed hemostasis, peripheral vascular disease etc. Therefore, an appropriate wound dressing is required to provide a template for better recruitment of cells during wound healing cascades.
In this experiment, a cost effective freeze dried scaffold was developed by combining tempo oxidized nanocellulose (TOCN) with chitosan using EDC/NHS as catalyst along with loading of antibacterial lawsone to achieve controlled release kinetics of this compound throughout healing periods.

METHODS:The TOCN (1%w/v) and chitosan (2.5, 3.5, 4.5, and 5.5 %w/v) were mixed followed by freeze drying. The porous sponge was immerged into EDC/NHS solution for certain time. The freeze dried scaffolds (TC2.5, TC3.5, TC4.5, and TC5.5) were then immerged in lawsone solution to facilitate crosslinking (TLC2.5, TLC3.5, TLC4.5, and TLC5.5).The physicochemical study involved morphology, porosity, confirmation of crosslinking. The in vitro degradation in acidic and physiologic pH, antibacterial assay, release pattern, cell viability and proliferation studies were conducted also. The in vivo wound healing performance of different samples was evaluated using full thickness excisional wound model in rats for 2 weeks.

RESULTS:The pore diameter decreased after increasing concentration of chitosan. Fourier transform infrared spectroscopy proved crosslinking of chitosan and TOCN. The degradation was faster in acidic environment (PH 6, PBS containing lysozyme) indicating that all these sponges except TLC5.5 group exhibited desirable degradation characteristics for in vivo application. The in vitro drug release behavior of sponge in pH 6 values of PBS indicated that these pH sensitive scaffolds released more lawsone in the acid environment which was adapted to physiological skin milieu and majority of wounds. The antibacterial properties of different samples were better while adding the lawsone in scaffolds.
Highest l929 fibroblast cell proliferation was found on TLC4.5 scaffolds after 7 days. The masson trichrome staining of tissue samples treated with different scaffolds displayed that wound closure and collagen deposition occurred highest in rats treated with TLC 4.5 scaffolds.

DISCUSSION & CONCLUSIONS:TOCN, Chitosan and Lawsone are ideal material for chemical modification. The granulation tissue thickness and relative gene and protein expression were significantly unregulated in rat treated with TLC4.5 scaffolds after 2 weeks of experiment concluding that TLC4.5 is the optimum scaffolds and potential for wound healing application.

Keywords: Drug delivery, Polymers - natural / synthetic / responsive



Regulation of corneal wound healing by the WNK1 kinase in a human tissue-engineered cornea

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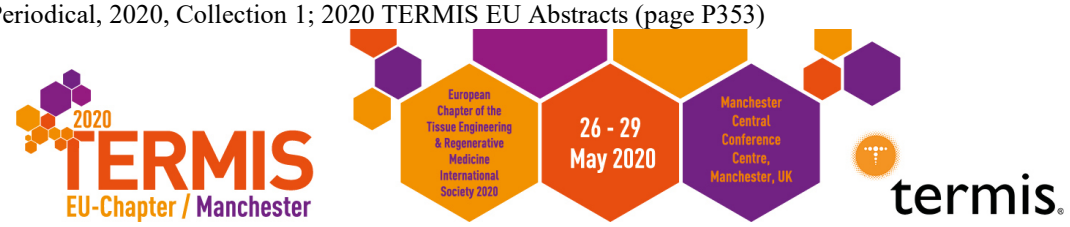
INTRODUCTION:Damage to the corneal epithelium triggers important changes in the composition of the extracellular matrix to which the basal human corneal epithelial cells (hCECs) attach. These changes are perceived by integrins, a family of trans-membrane receptors that activate different intracellular signalling pathways, ultimately leading to re-epithelialization of the injured epithelium. Among the intermediates downstream of integrin signalling, WNK1 is the kinase whose activity is the most strongly increased during corneal wound healing. The goal of the present study was to determine the contribution of WNK1 to corneal wound healing and to identify the mechanisms and proteins through which WNK1 mediates its influence on that process.

METHODS:A scratch wound assay conducted on hCECs grown as monolayers and a wound healing protocol performed on tissue-engineered human corneas (hTECs) were both used as in vitro models to study the impact of a WNK1 pharmacological inhibitor, WNK463, on the migratory and proliferative properties of hCECs, on the organization of the actin cytoskeleton and corneal wound closure. Gene profiling analyses and transcription factors analyses were also conducted either in the presence or absence of WNK463.

RESULTS:WNK1 inhibition by WNK463 altered the migratory and proliferative properties of hCECs and significantly reduced the rate of corneal wound closure in monolayer and in our 3D model. It also prevented the activation of its downstream target proteins SPAK and OSR1 that are expected to contribute to the healing mechanism. In addition, WNK1 inhibition also modified the gene expression pattern in hCECs and considerably reduced the activity and expression of a number of transcription factors in vitro.

DISCUSSION & CONCLUSIONS:These results will contribute to a better understanding of the cellular and molecular mechanisms involved in corneal wound healing. Furthermore, they will validate a new function for the WNK1 kinase in corneal wound healing and might lead to the identification of a new therapeutic target in the field of corneal wounds.

Keywords: Wound healing, Eye



Proteins in body fluids change the antiadhesive properties of HA-based foils

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INTRODUCTION:Physical barriers can prevent postoperative adhesion in abdominal cavity. Hyaluronic acid (HA) is one of the main studied component thanks to its anti-adhesive properties with positive effect on wound healing1. HA-based foils are commercially available; however, their usage is not 100% effective with several side effects. One of the reasons could be the interaction of the material with proteins present in body fluids. The aim of this study was to monitor the influence of protein sorption on antiadhesive properties of HA-based foil barriers.

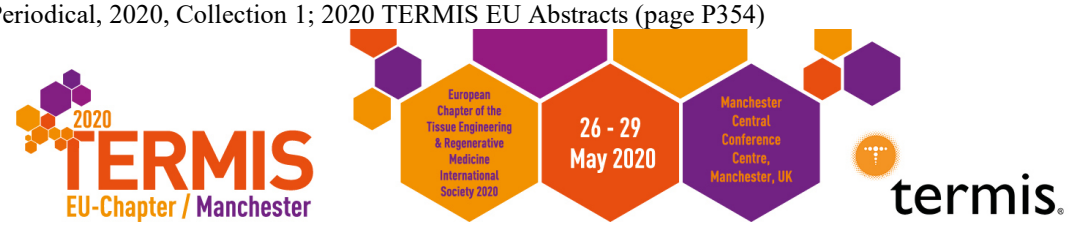
METHODS:The different foils based on HA (HA + carboxymethylcellulose, benzyl-ester of HA) were exposed to fetal bovine serum or mildly heparinized (3 IU/ml) human blood for 15 min and 2 hrs. The concentration of protein sorption on the material was measured by BCA protein assay (Thermo Fisher Scientific). The quantity of proteins was compared to samples implanted in mouse peritoneum. The adhesion of NHDF cells pre-stained by fluorescent DiI were then monitored under the confocal microscope after 24 hrs.

RESULTS:The presence of blood proteins, but not serum or cultivation medium (control) highly changes the properties of antiadhesive foils. The concentration of proteins was 0 for cultivation medium, low for FBS (ca. 0.005 mg/sample) and medium (ca. 0.07 mg/sample) for blood after 15 min without differences between foils. With longer treatment, there was a massive protein sorption from blood only on samples made of HA + carboxymethylcellulose (0.3 mg/sample). This sample was also the most unstable during handling. The subsequent test of cellular adhesion supported these results when blood-treated antiadhesive foils enabled the adhesion of NHDF cells.

DISCUSSION & CONCLUSIONS:The presence of blood in abdomen during the surgery could be the source of adhesive proteins, especially fibrinogen, which cover the antiadhesive barrier. This changes its primary function to prevent the adhesion and leads to proadherent surface. Based on our research, the antiadhesivity of material should be controlled in vitro before the animal study not only as a raw material, but also after pretreatment with protein-rich body fluids.

References:1) Reijnen et al., Br. J. Surg. 2003; 90(5):533-41

Keywords: Biomaterials



bFGF loaded Heparinized PCL/gelatin co-spun nanofabrics as growth factor delivery vehicle for accelerated wound healing

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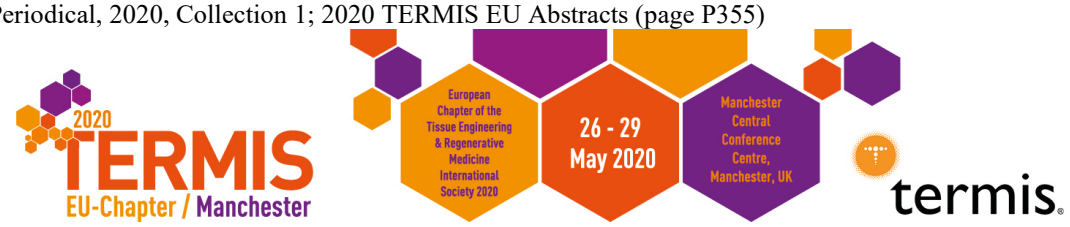
INTRODUCTION:Growth factor (GF) incorporation to tissue engineered scaffolds is an important aspect to be considered for mimicking the complex in- vivo conditions. Low stability and early denaturation of such signalling molecules due to proteolytic degradation under physiological condition limits their effective use in tissue engineered scaffolds. Heparin functionalized surfaces has been widely used by researchers as growth factor delivery vehicles due to well-known property of heparin to stabilize and facilitate the interaction of several growth factors with the scaffold material through electrostatic interactions. The aim of this research is to fabricate PCL/Heparin conjugated gelatin core-shell type nanofibers and to evaluate their efficacy to load growth factors and their effectiveness in delivering GF by performing in-vitro and in-vivo evaluations. Our studies revealed that fabricated nanofibers showed improved mechanical properties; high heparin content and high efficiency to immobilize basic fibroblast growth factor (bFGF). In-vitro evaluation by fibroblast culture revealed nanofibers showed high viability for cultured cells and promoted spreading morphology. In-vivo; full thickness wounds treated using nanofibers showed accelerated wound closure, minimum scar formation and generation of skin appendages. From our findings; we can conclude high potential of such nanofabrics as GF delivery systems for wound healing applications.

METHODS:Using co-axial electrospinning technique, PCL/gelatin core-shell type nanofibers were produced and were conjugated to heparin using EDC/NHS coupling. Synthesized heparinized membranes were then loaded with bFGF to improve there curing tendency. Full thickness wound rat model was then treated using synthesized nanofibers to analyze their effectiveness in promoting wound healing.

RESULTS:From in vitro-evaluations; bFGF loaded nanofibers promoted high cell viability and cell spreading for cultured cells under low serum condition. Evaluation of wounds by histological examination, the skin regeneration was scar free with generation of skin appendages.

DISCUSSION & CONCLUSIONS:ECM mimicking nanofibers are often poor in mechanical properties. To overcome this drawback; this research employs the use of co-electrospinning technique which can produce core-shell fiber with improved mechanical properties as well as low degradation rate. Also; since use of nanofibers alone may not accelerate the complex process of healing; we employed heparin to the fabricated nanofibers and bFGF loading was done to enhance their curing tendency due to well-known tendency of bFGF to promote accelerated and scar-less healing. From wound appearance as healing progressed and histological examinations; our nanofiber matrices were superior to not only accelerate the process; but to also ensure scar less tissue regeneration.

Keywords: Nanomaterials (inc graphene), Biologics and growth factors



A novel model of digit injury and regeneration in mice

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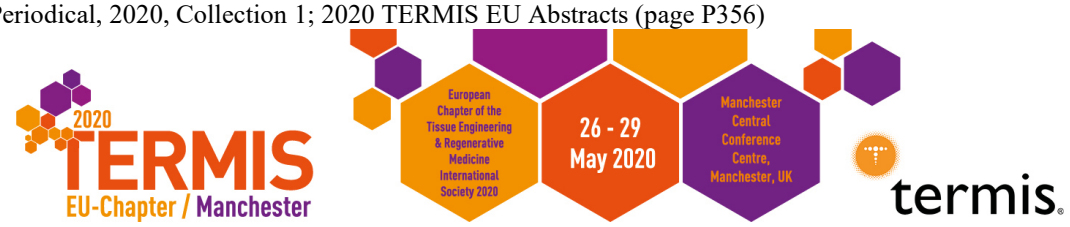
INTRODUCTION:Traumatic injuries of the hand are a common clinical problem, with high morbidity. Loss of function in the hand through tissue fibrosis severely impacts a patient's quality of life; therefore, stimulating regeneration of hand tissues is a critical objective. Achieving this is difficult, because the hand comprises of multiple distinct tissue types designed to move freely, making the healing process complex. A particularly extreme example of this is degloving injuries resulting from ring avulsion, where one or more tissues are stripped from the digit. Animal models of hand injuries are scarce, and previous literature has yielded no animal models of composite tissue injuries. We describe a new mouse model of this injury and assess its utility in comparing wildtype and regenerative mouse phenotypes.

METHODS:An observational study of a microsurgical murine composite tissue injury of the digit in C57/Bl6 (wildtype) mice was performed and assessed for healing over 42 days. The injury resembling a ring avulsion injury involved the neurovascular bundle, tendon and skin were excised between the distal interphalangeal joint and the proximal digital crease. A further comparative study of 12 C57/BL mice (wildtype) and 12 MRL/MpJ (regenerative) phenotype were compared for healing and concordant ear punches were made to assess the regenerative capacity of the mouse during their healing. Tissue was harvested at day 0, 7, 21, and 42 in the observational study and at 6 and 20 days in the comparison study. Digits were decalcified in EDTA. Immunohistochemical staining for markers of proliferation (BrdU), Collagen synthesis (Hsp47), Pericytes and myofibroblasts (αSMA) and basement membrane (Laminin) was undertaken.

RESULTS:The observational study in C57/Bl6 mice showed a thickened epithelial layer had formed over the wound at day 7, negative for the basement membrane protein laminin. At day 21, laminin was detected and there were significantly more subcutaneous type I collagen production from cells indicating fibrosis. No regenerative characteristics were observed although healing and fibrosis was evident. MRL/MpJ mice healed more rapidly with evidence of early more prevalent angiogenesis. Signs of "blastema" formation were observed suggesting greater regenerative potential.

DISCUSSION & CONCLUSIONS:We have developed a model for severe traumatic injury to the mouse digit involving a number of different tissue types. We describe another example where the MRL/MpJ mouse shows improved regenerative characteristics. Future investigation of the molecular differences between the two mouse groups may identify cues for better healing of such injuries in mammalian systems.

Keywords: In vivo and animal models,



Developing a Delivery System for Cell Therapy Treatment of Chronic Wounds using Human Dermal Fibroblasts and Bone Marrow- Mesenchymal Stromal Cells

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INTRODUCTION:Pressure ulcers (PrU) are a major clinical challenge following spinal cord injury. The treatment of a PrU using a commercial allogeneic acellular dermis (Allosource®), developed for diabetic chronic wound treatment, has been tested at our hospital. This study aimed to assess the biocompatibility of Allosource® with human juvenile dermal fibroblasts (DFs) and human adult bone marrow mesenchymal stromal cells (BM-MSCs).

METHODS:DFs (n=3) were isolated from surgically removed digits via enzymatic separation of the dermis and epidermis (Dispase II), followed by collagenase II (Worthingtons, USA) digestion of the dermis or explant culture. BM-MSCs were isolated from young, healthy donors (Lonza; n=3).

RESULTS:The population doubling time for digested DFs, explant cultures and BM-MSCs was 3.9, 1.4 and 2.0 days, respectively. Following inflammatory stimulation (interferon-γ and lipopolysaccharide), HLA-DR, CD40, CD80 and CD86 were not detectable by flow cytometry in DFs or BM-MSCs. DFs were seeded onto 5mm2 AD explants in 100% FCS (n=3 per condition) for 20mins to simulate intra-operative delivery of cells or for 1 hour to test the effect of an extended adhesion time. Tissue engineered ADs were cultured for 2 weeks at 370C at 5% CO2. After 48h in culture, lactate levels of 1.6, 1.9, 1.9 and 2.2mmol/l were measured in the conditioned medium from DF seeded grafts seeded with no cells, 10K, 20K, and 50K, respectively. After 2 weeks DFs aligned parallel to the surface of the dermis (H&E stained histological sections; Figure 1). After 2 weeks in culture cytotoxicity assays indicated a mean cell number of 35.0K and 68.5K for 20 min and 1hr adhesion grafts, respectively.

DISCUSSION & CONCLUSIONS:Our preliminary analyses indicate that the AD (Allosource®) is biocompatible with juvenile DF and adult BM-MSCs. DFs differentiate into myofibroblasts during wound closure producing ECM proteins. BM-MSCs have established anti-inflammatory and paracrine functions that promote wound healing. ADs have potential as a cell delivery system for therapeutic cell populations that might promote healing of chronic wounds.

ACKNOWLEDGEMENTS:Allosource for the provision of acellular dermis samples for research.

Keywords: Cell therapy, Wound healing

Electrospun Poly(Ɛ-Caprolactone)/Collagen NanofibrousScaffolds Support Growth Factor and Extracelular Matrix Protein Production by Wharton Jelly Mesenchymal Stromal Cells

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INTRODUCTION:Current treatments for restoration of skin damage are limited to the availability of healthy autologous grafts or allogeneic skin donors. Despite the progress in engineering dermal substitutes, most available scaffolds are restricted only to shield the wound.Thus, the need to create scaffolds that provide coverage and accelerate tissue repair processes in vivo is warranted.

METHODS:In this study, poly(e-caprolactone)/collagen type I (PCL/COL) scaffolds were manufactured in a 19:1 ratio polyester: protein using electrospinning, as an alternative approach to generate bioactive scaffolds for skin regeneration. The obtained scaffolds were characterized by analytical techniques such as SEM, contact angle, FT-IR, TGA, DSC and mechanical properties. Finally, biocompatibility of PCL/COL scaffolds was tested by seedingWharton's jelly mesenchymal stromal cells (WJ-MSC). Biological functions such as cell adhesion, proliferation and production of growth factor/extracellular matrix proteins (KGF, b-FGF, collagen II y III) was further examined.

RESULTS:Results demonstrated that addition of only small quantities of collagen in PCL/COL scaffolds decreased fiber diameter and Young's modulus, increased wettability and supported WJ-MSC adhesion and proliferation. Moreover, PCL/COL scaffolds triggered the production of growth factors and extracellular matrix proteins by WJ- MSC.

DISCUSSION & CONCLUSIONS:Thus, the inclusion of small amounts of collagen might prevent significant changes in the chemical and biological characteristics associated to large amounts of collagen. Importantly, PCL/COL scaffolds obtained proved to have advantageous mechanical and biological properties that make them promising candidates for skin tissue engineering.

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Keywords: Biomaterials, Polymers - natural / synthetic / responsive