Meeting of the Glasgow Orthopaedic Research Initiative

Tuesday 21st March 2023

Advanced Research Centre | University of Glasgow | 11 Chapel Lane | Glasgow | G11 6EW

08:45 **Registration/coffee**

PROGRAMME

Session 1

(chaired by David Shields & Emma Kelly)

09:15 **Welcome**
Professor Dominic Meek & Professor Matt Dalby

09:20 **Current paradigms of care in critical sized bone defects**
Dr Bilal Jamal, NHS Greater Glasgow & Clyde

10:00 **Trabecular Metal collars in endoprosthetic replacements: do they osteointegrate?**
Dr Ewen Fraser, Glasgow Royal Infirmary

10:20 **Student research talks**

Seb Doherty-Boyd: Developing an artificial bone marrow niche for HSC maintenance and genetic manipulation

Egle Morta Antanaviciute: Nanovibrational stimulation affects oxidative phosphorylation and reactive oxygen species scavenging in human dermal fibroblasts

10:40 **Tea/coffee**

Session 2

(chaired by Marta Cubero Sarabia & Nadia Soulioti)

11:00 **Joint instability: an engineering perspective**
Dr Philip Riches, University of Strathclyde

11:30 **Finite element modelling of tibial defects managed with a fine wire fixator**
Dr David Shields, Queen Elizabeth University Hospital

11:50 **Stem cell chondrogenesis can be regulated by the viscous component of isoelastic matrices**
Dr Matthew Walker, University of Glasgow

12:10 **In-vitro studies of 2D orthopaedic implants with cell instructive nanotopographies**
Dr Rosalia Cuahtecontzi Delint, University of Glasgow

12:30 **Sponsor presentations**
Biocomposites - Christopher Connell (3-5 mins)

ThermoFisher - Selina Henriquez (3-5 mins)

12:40 Posters and lunch

Session 3

(chaired by Rosalia Cuahtecontzi Delint and & Ian Kennedy)

13:30 Controlled release antimicrobial Sol-gel coatings to prevent prosthetic joint infection
Dr Tim Nichol, Sheffield Hallam University

14:00 Bioengineering of a model of the bone marrow niche to investigate the mechanical changes in the microenvironment
Dr Chloe Rodgers University of Glasgow

14:20 Deciphering the role of boron transporter, NaBC1, on mechanotransduction mechanisms
Dr Juan Gonzalez-Valdivieso University of Glasgow

14:40 Outcomes of dual mobility total hip replacements for neck of femur fractures
Matthew Arnold, West of Scotland Trauma and Orthopaedics

15:00 Student research talks

Amina Rezig: Investigating the application of perylenebisimide hydrogels as a tissue engineering scaffold

Sarah Eccles: In vitro characterisation of a polymer-rifampicin coating for prevention of orthopaedic implant infections

Mariana Azevedo Gonzalez Oliva: The role of Piezo1 in transducing matrix viscoelasticity

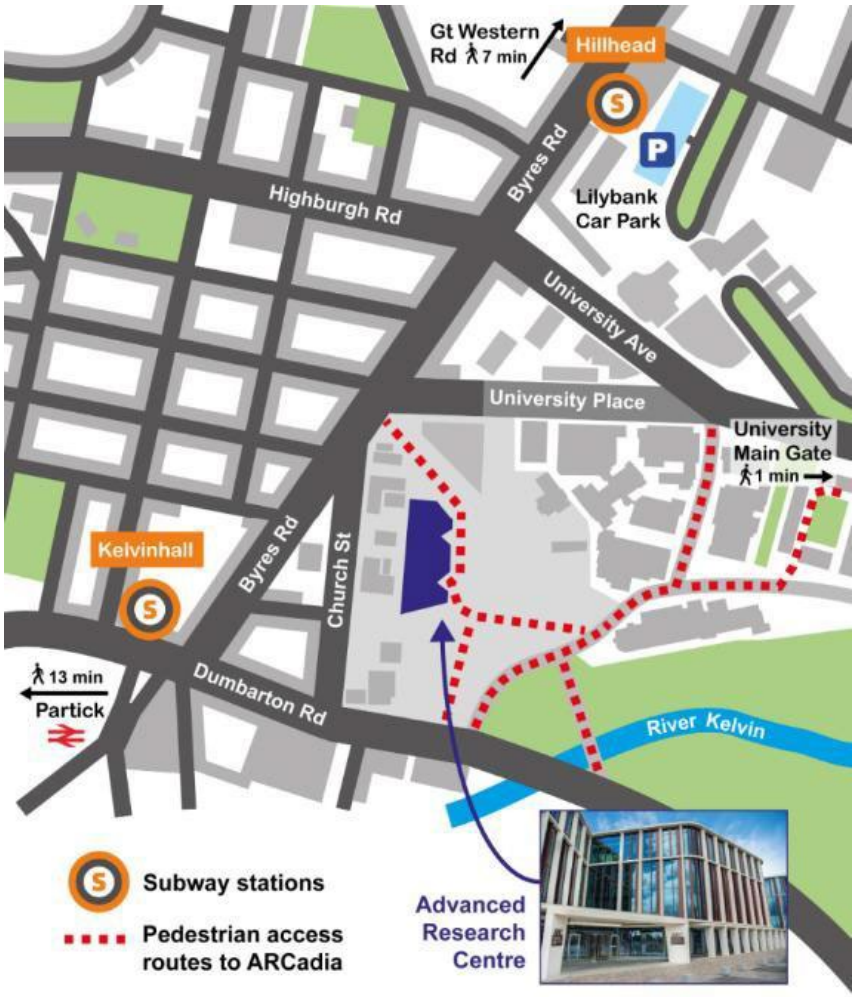
Lauren Hope: Engineering a bone marrow endosteal niche model for drug screening against Acute Myeloid Leukaemia

15:40 Prizes

16:00 Meeting ends

DIRECTIONS

The best subway to take is to Hillhead. On leaving Hillhead subway, go left down Byres Road and then left to University place and you will find pedestrian access to the ARC building. On the image below you can see the pedestrian access routes. You can also find us on google maps.



ORAL PRESENTATIONS

Current paradigms of care in critical sized bone defects

Dr Bilal Jamal

NHS Greater Glasgow and Clyde

Trabecular Metal collars in Endoprosthetic Replacements: do they osteointegrate?

E. Fraser, S. Spence, O. Alanie, J. Doonan, H. Findlay, A. Mahendra, S. Gupta

Glasgow Royal Infirmary

Background

Limb salvage surgery (LSS) is the primary treatment option for primary bone malignancy. It involves the removal of bone and tissue followed by reconstruction to save a limb and prevent amputation. Endoprosthetic replacements (EPRs) are used for LSS, and Trabecular Metal (TM) collars have been developed to encourage bone ingrowth (osteointegration) into EPRs. Several studies were identified that looked at various types of collar material in EPRs for a bone tumour. No studies, however, were found that looked solely at TM collars in EPRs. The primary aim of this study was to assess whether osteointegration occurs when TM collars are used in EPRs for tumour.

Methods

All patients (n=124) from 2010-2021 who underwent an EPR for tumour under the West of Scotland orthopaedic oncology team were identified. 65% (n=81) of patients met the inclusion criteria and two consultants independently analysed radiographs at 3 months, 12 months, and last x-ray using the Stanford Radiographic Assessment System (SRAS). Interobserver reliability was also assessed.

Results

Osteointegration of the TM collar was found to have occurred in approximately 65% of patients at last x-ray. The percentage of patients with osteointegration at 3 months (65.4%) reflected 12 months (65%) and last x-ray (64.4%). Radiolucency at the bone:collar junction was present in 28.4% (n=23) of cases at 3 months but only 6.7% (n=4) showed progression of this at 12 months. The interobserver reliability was found to be highly reliable in all parameters ($p<0.001$).

Conclusion

Osteointegration occurs in TM collars but at rates lower than that of Hydroxyapatite for the same purpose. Osteointegration will occur by 3 months and will reflect osteointegration at 12 months and last x-ray. Furthermore, radiolucency at the bone:collar impact junction does occur in some patients but only a low number will show radiolucency progression at longer term follow-up.

Developing an artificial bone marrow niche for HSC maintenance and genetic manipulation

W. Sebastian Doherty-Boyd[2], Adam West[2], Hannah Donnelly[2], Monica Tsimbouri[2], Manuel Salmeron-Sanchez[2], Aline Miller[1], Matthew J. Dalby[2]

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Haematopoietic stem cells (HSCs) are responsible for the process of haematopoiesis, the continuous production of blood and immune cells (Seita & Weissman, 2010). HSCs natively reside within the bone marrow (BM) niche, which maintains the stem cell pool and haematopoietic activity with a variety of stimuli (Wei & Frenette, 2018). Research on HSCs and the BM niche could improve our understanding of the causes and treatments for BM-associated disorders (Frangoul et al., 2021) and enable HSC expansion in vitro, to meet the demand created by BM transplants and HSC research (Kumar & Geiger, 2018).

HSCs cultured under standard conditions in vitro differentiate rapidly, hampering research (Schoffield, 1978). The solution is the creation of a reliable system capable of maintaining HSCs ex vivo by mimicking aspects of the BM niche (Bello et al., 2018). My project aims to develop such a system centred around a synthetic, peptide-based hydrogel.

Our system will use mesenchymal stem cells (MSCs) as a feeder layer. The MSCs will be cultured on a surface coated with poly(ethyl acrylate), a monomer that causes fibronectin, which the surface will also be coated with, to assemble in an open conformation (Llopis-Hernandez et al., 2016). This allows the fibronectin molecules RGD and growth factor binding domains, which will be loaded with the osteogenic growth factor BMP2, to synergistically signal the MSCs cultured on top of them. A peptide-based hydrogel will also be layered on top of the MSCs. This system will mimic some of the biochemical and mechanical properties of the BM niche, encouraging the MSCs to adopt a niche-like phenotype (Donnelly, 2020). As MSCs natively reside alongside HSCs in the BM niche and are thought to provide various paracrine signals to them (Pinho and Frenette, 2019), the MSCs in our system will promote the maintenance of HSCs cultured on top of the gel.

Nanovibrational stimulation affects oxidative phosphorylation and reactive oxygen species scavenging in human dermal fibroblasts

E. M. Antanaviciute, P. M. Tsimbouri, M. J. Dalby

Centre for the Cellular Microenvironment, The University of Glasgow

Vibration of nanoscale amplitude delivered at a high frequency (1000Hz) has been shown to promote mesenchymal stem cell osteogenesis even in the absence of biochemical cell fate inducers. This suggests that nanovibrational stimulation could be applied as mechanotherapy to supplement or replace pharmacological treatment for patients with osteoporosis or slow-healing bone fractures. However, it is largely unknown how cells in the soft tissues around the bone may respond to this type of stimulation. Fibroblasts are the most abundant cell type in the soft tissues. They are responsive to various mechanical stimuli, which can alter their fibrotic or wound healing properties. Metabolomic and transcriptomic analyses were carried out to investigate dermal fibroblast response to nanovibrational stimulation. The results indicate that nanovibration promotes oxidative phosphorylation while also promoting reactive oxygen species scavenging, enabling cell growth and proliferation while potentially suppressing fibrogenesis.

Joint instability: an engineering perspective

Dr Philip Riches

Biomedical Engineering, University of Strathclyde

Joint instability, in which there is a perceived lack of confidence regarding the mechanical functioning of a joint whilst is it loaded, is prevalent in both knees and ankles. Knee joint instability occurs in osteoarthritic (OA) and TKA populations, the latter accounting for ~17% of all revisions, whilst chronic ankle instability is a widespread problem specifically across athletic populations. Patient-reported outcome measures, for example the Oxford Knee Score, Knee Injury and Osteoarthritis Outcome Score and the Cumberland Ankle Instability Tool, provide a qualitative, perceived measure of a potentially measurable, mechanical dysfunction. This talk will explore approaches to developing a quantitative biomechanical biomarker of instability, using gait variability, resonant frequencies and even chaos theory, which have been applied to OA, TKA and athletic populations.

Finite Element Modelling of Tibial Defects Managed with a Fine Wire Fixator

Mr David Shields[1], Dr Karol Lewandowski[2], Dr Andrew McBride[2], Dr Lukasz Kaczmarczyk[2], Mr Bilal Jamal[1]

[1]*Queen Elizabeth University Hospital, Glasgow, United Kingdom*
[2]*University of Glasgow, Glasgow, United Kingdom*

Introduction

Circular frame fixation has become a cornerstone of non-union and deformity management since its inception in the 1950s. As a consequence of modularity and heterogenous patient and injury factors, the prediction of the mechanobiological environment within a defect is subject to wide variations in practice. Given these wide range of confounding variables, clinical and cadaveric experimentation is close to impossible and frame constructs are based upon clinician experience. The Finite Element Analysis (FEA) method provides a powerful tool to numerically analyse mechanics. This work aims to develop an FEA model of a tibial defect and predict the mechanical response within the construct.

Materials and Methods

The geometry of a tibia was acquired via CT and a series of bone defects were digitally created in the tibial diaphysis. A 4-ring, 10-wire Ilizarov fixator was constructed using 180mm stainless steel rings and 1.8mm stainless steel wires tensioned to 1200N. An axial load (800N) was applied to simulate single leg stance of an 80kg patient. The magnitude of displacement was measured for defects with varying sizes (5-40mm). A numerical analysis was performed in large-strain regime using open-source FEA library (MoFEM).

Results

Defect size did not effect displacement, but significantly influenced strain. Measured displacements were 5.72-5.78mm, however strain ranged from 14.5-100%. Moreover, it was found that bone material properties also have no significant impact on the results.

Conclusions

Accounting for FEA assumptions, this model predicted a strain environment which was above expected favourable range for bone healing. The addition of graft within the environment is likely to change the mechanobiological environment which warrants further investigation. We plan to develop this model to answer further research questions in the limb reconstruction discipline and validate its accuracy with mechanical data. We believe the presented approach can be a useful tool for investigating the performance circular frames.

Stem cell chondrogenesis can be regulated by the viscous component of isoelastic matrices

Matthew Walker, Eonan William Pringle, Manlio Tassieri, Delphine Gourdon, Marco Cantini

Centre for the Cellular Microenvironment, University of Glasgow

Hydrogels are cost effective and widely used extracellular matrix (ECM) models with tuneable biochemical and mechanical properties. Their viscous character has been shown to tune adhesive and mechanotransductive signalling pathways, ultimately regulating stem cell differentiation. However, the design of most hydrogels disregards the viscous nature of native ECMs, focussing only on their elastic properties. In the case of stem cell chondrogenesis, this has led to contradictory results due to interference from other factors, e.g., biochemical cues or indeed unreported changes of the matrices' viscous modulus. Here, by employing isoelastic matrices with a Youngs' modulus of circa 12 kPa, we demonstrate that variations in the viscous properties (i.e., with loss tangent varying between 0.1-0.25) of 'RGD-functionalised polyacrylamide' or 'polyethylene glycol maleimide' hydrogels drive efficient chondrogenesis of human mesenchymal stem cells, both in 2D and 3D cultures. In particular, the increase of the viscous component of isoelastic hydrogels promotes a phenotype with reduced spreading and adhesion, altered mechanosensitive signalling, and increased cell-cell contacts; this, in turn, upregulates the chondrogenic transcription factor SOX9, supporting neocartilage formation.

In-vitro studies of 2D orthopaedic implants with cell instructive nanotopographies

R. Cuahtecontzi Delint[1], M. P. Tsimbouri[1], I. Ishak[2], V. Jayawarna[1], A. Nobbs[2], M. Salmeron-Sanchez[1], K. Burgess[3], B. Su[2], & M. J. Dalby[1]

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[3]School of Biological Sciences, University of Edinburgh

Demand for total hip (THR) and knee replacements (TKR) is increasing worldwide, due to degeneration of subchondral bone, a result of ageing populations and active lifestyles. While the success rate of these implants is relatively high, many fail within 10 years. The two leading causes are aseptic loosening (due to poor osseointegration) or infection (due to bacterial biofilm formation). Although certain topographies are bactericidal, they do not support osteogenesis on their own and require protein coatings. Here, we use plasma poly(ethyl acrylate) (pPEA) polymer coating on surfaces to spontaneously organise fibronectin (FN) and deliver bone morphogenetic protein-2 (BMP2) to improve human mesenchymal stem cells (hMSCs), that have bone forming potential, adhesion and osteogenesis. Simultaneously, the designed patterning and pPEA+FN+BMP2 active coatings have been developed to reduce biofilm formation(1).

Nano-patterned titanium (Ti) surfaces were prepared by etching in sodium hydroxide for 2h or 16h producing nanosurface 1 (NS1) and nanosurface (NS2), yielding nanospike structures of 400 and 500 nm in height, respectively that could be plasma coated with PEA+FN+BMP2. The viability and adhesion quality of primary hMSCs on the coated and uncoated Ti topgraphies was tested using immunofluorescence staining and colourimetric assays. Antimicrobial properties of coated and uncoated surfaces were challenged using Gram-stained negative bacteria Pseudomonas aeruginosa. The viability of hMSCs co-cultured with Pseudomonas aeruginosa on titanium surfaces was evaluated, as well as the hMSCs attachment to the titanium surfaces and their osteogenic differentiation potential.

Nanopatterned titanium surfaces treated with active coatings such as PEA+FN+BMP2 offer great potential to grow viable hMSCs and sustain osteogenic differentiation. Furthermore, both topographies showed indication of antibacterial potential, corresponding with previous studies prepared using high aspect ratio nanospikes.(2) Moreover, the exposure of hMSCs to bacteria caused no significant change in the hMSC viability or adhesion features in short-term co-culture. Ultimately, conditions were more favourable for the titanium surfaces with nanopatterns.

This study showed the potential for the implementation of nanotopographical surfaces to address poor osseointegration on bone implants and bacterial biofilm formation after THR and TKR. Coated topographies offer better support for hMSC adhesion and differentiation versus uncoated surfaces. This finding is of importance given that primary cells need to be recruited for successful osseointegration. Moreover, biofilm formation was decreased when bacteria was seeded on NS1 and NS2 patterned surfaces, aiding hMSC colonisation onto the titanium surface.

REFERENCES

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Controlled release antimicrobial Sol-gel coatings to prevent prosthetic joint infection

Tim Nichol

Department of Biosciences and Chemistry, Sheffield Hallam University

Periprosthetic joint infection (PJI) is a major complication of arthroplasty surgery, with 1-3% of all primary procedures and up to 15% of revision procedures resulting in infection. Effective treatment typically involves local delivery of antibiotics via antibiotic-loaded bone cement, as well as systemic antibiotic delivery. However, over the past 20 years there has been an increasing trend of cementless procedures which removes the ability to provide local antibiotic release. Here we present the development of a thin film sol gel coating technology for prosthetic devices, which can be used for controlled release of antimicrobial agents and can provide local antimicrobial delivery for cementless procedures. The coating can be applied to surfaces in a variety of ways such as spray coated, dip coated or painted on, and has been tested against a range of antibiotics, antifungals, biocides etc. By altering the formulation we have demonstrated controlled release from hours to days to weeks. These coatings demonstrate strong antimicrobial activity against a range of clinically relevant organisms, show no adverse bone healing in an in vivo rat model and a favourable cytotoxicity profile against a range of human cells. We propose that this technology has the potential to become a powerful tool in the prevention and treatment of prosthetic joint infection.

Bioengineering of a model of the bone marrow niche to investigate the mechanical changes in the microenvironment

Chloe Rodgers, Eva Barcelona-Estaje, Oana Dobre, Manuel Salmeron-Sanchez, Matthew Dalby

Centre for the Cellular Microenvironment, University of Glasgow

The bone marrow niche contains a multitude of cell types and a microenvironment supportive of haematopoiesis. Mesenchymal stem cells (MSCs) secrete various growth factors which support the maintenance of haematopoietic stem cells (HSCs) in a quiescent state. Similarly, leukemic stem cells rely on these interactions in the bone marrow niche to support their survival and resistance to treatment. Improvements in the culturing of HSCs and MSCs in vitro have involved (1) the development of 3D culture models; (2) the co-culture of the two cell types; and (3) the addition of mechanical stimulation.

Here, we have designed and optimised a system to recapitulate the 3D microenvironment of MSCs and HSCs by using synthetic fibronectin-based hydrogels in which the stiffness and degradability can be tuned. Mechanical cues have an underappreciated role in fostering the correct functioning and survival of cells, therefore, the effect of applying mechanical stimulation to the model niche was explored by applying vibrations of nanoscale amplitude and measuring the bulk and local mechanical properties of the hydrogels.

We show that MSCs, HSCs and LSCs are viable when cultured in our 3D model of the bone marrow niche. Further, we demonstrate that HSCs and leukemic stem cells respond differently to mechanical stimulation. In conclusion, we have developed a method for the 3D culture of the bone marrow niche incorporating both HSCs and MSCs which can be utilized to investigate the mechanical changes in cell and ECM stiffness in response to malignant cells.

Deciphering the role of boron transporter, NaBC1, on mechanotransduction mechanisms

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[3]*Bioengineering, Biomaterials and Nanomedicine Networking Biomedical Research (CIBER-BBN)*

More than 500 neuromuscular disorders affect about 750.000 patients in Europe and the US, who suffer from progressive muscle wasting leading to poor quality of life and reduced life expectancy. Muscular dystrophies are rare, terminal disorders that affect muscle cells by progressive wasting and weakness. Patients are unable to repair the continuous muscle damage and eventually lose muscle tissue and function, resulting in disability and early mortality. The cell matrix interactions are crucial for normal biological processes and their disruption can lead to pathological processes. In particular, the biological importance of extracellular matrix (ECM) cytoskeleton interactions in skeletal muscle is accentuated by the number of muscle diseases caused by defects in proteins involved in these interactions, considered as mechanotransduction disorders.

The aim of our work is based on the functional coupling of different cell membrane receptors: fibronectin (fn)-binding integrins and NaBC1 boron transporter. We engineer fn-functionalised 3D PAAm hydrogels with different stiffness (from 0.5 to 35 kPa). Muscle cells are cultured onto the different substrates in the presence/absence of soluble Boron to evaluate myogenic differentiation and its correlation with the active NaBC1/Fn-binding integrin cluster and the different rigidities. We also evaluate whether the different stiffness of substrates affected the effect of Boron in cell features and behaviour regarding cell adhesion, spreading, elasticity or signalling pathways.

Our results show that simultaneous NaBC1-integrin cooperation enhances intracellular signalling and may compensate the altered cell-ECM interactions in mechanotransduction disorders. Muscle cells are shown to better attach to stiffer substrates, where the cell adhesion and spreading is enhanced by the presence of Boron in a concentration-dependent trend. Moreover, the simultaneous stimulation of NaBC1 and FN-binding integrins enhance myotube formation in dependence of the substrate stiffness.

In conclusion, PAAm hydrogels with different mechanical properties are a useful biomaterial tool to study mechanotransduction mechanisms. Our findings show that the simultaneous stimulation of NaBC1 and fibronectin-binding integrins trigger in vitro myogenic formation in dependence of the substrate stiffness, suggesting a new role for NaBC1 transporter besides controlling B homeostasis, acting as a mechanoresponsive protein.

Outcomes of dual mobility total hip replacements for neck of femur fractures

M.Arnold, J. Kennedy, E. Wright, M. Reece, RMD. Meek

West of Scotland Trauma and Orthopaedics

Background

Neck of femur fractures are extremely common worldwide and have a mortality rate of 15% after 1 year. Dual-mobility cups (DMCs) have demonstrated a reduction in dislocation and revision rates for elective total hip arthroplasty (THA) but the choice to use DMCs for neck of femur fractures is less clear. The aim of this study was to compare the rate of dislocation for patients who received a conventional THA to those that received a DMC for a neck of femur fracture.

Methods

Data was retrospectively collected on patients who received either DMCs or standard cups for neck of femur fractures at Queen Elizabeth University Hospital Glasgow. Patients were chosen to allow for a 2 year follow up. Complications for each patient group were collected and the dislocation rates between the two implants were then compared to one another.

Results

Data was collected on 39 patients with DMCs and 95 patients with conventional cups. 2 patients with DMCs suffered a dislocation (5.1%) compared to 7 patients (7.3%) who underwent a conventional THA ($p = 0.49$). 1 DMC patient required revision surgery and one conventional THA patient underwent a revision for aspetic loosening.

Conclusion

Our study showed an insignificant reduction in dislocation rates in patients treated with a DMC compared to conventional THAs. Limitations include being a retrospective study and discrepancy in patient numbers between the two groups. Further studies are required to measure long term outcomes of DMCs, and it is also important to consider the increased cost of DMCs when choosing an implant.

Investigating the Application of Perylenebisimide Hydrogels as a Tissue Engineering Scaffold

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Perylenebisimide is a well-known member of the Rylene dyes family. This class of compounds is among the most promising n-type organic semiconducting materials due to their remarkable optoelectronic properties and excellent chemical/thermal stability. However, the research about their application in the biological field is scarce due to their hydrophobic nature. One way to obtain water solubility is by introducing hydrophilic substituents either to the core molecule or the imide position thus expanding their use in biological applications. This research explores, in a chemical and biological depth, the optimization of an Alginate hydrogel incorporating water-soluble amino acid functionalized Perylenebisimide (PBI), to create a conductive 3D network that facilitates the regeneration of electrogenic cells. In this work, we discuss the feasibility of PBI chemical synthesis and investigate the use of different rheological techniques to characterize the overall bulk properties of the gel to achieve mechanical tunability. We also look at their electrical properties and investigate the relationship of the gelator structures to the homogeneity of the gel network. Finally, we investigate their biocompatibility with myogenic cells in a structural and a concentration-based manner.

In vitro characterisation of a polymer-rifampicin coating for prevention of orthopaedic implant infections

Sarah Eccles[1], David W. Shields[2], Sean McGinty[3], Michelle Maclean[4], Christopher McCormick[1]

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[2]*Department of Trauma and Orthopaedics, Queen Elizabeth University Hospital*
[3]*Division of Biomedical Engineering, University of Glasgow*
[4]*The Robertson Trust Laboratory for Electronic Sterilisation Technologies, University of Strathclyde*

Introduction

Periprosthetic implant infection is a serious complication which can develop weeks to months after orthopaedic surgery. Since resistant infection is caused by notoriously difficult to treat bacterial biofilms, revision surgery is often required. Therefore, prevention is key to addressing this challenge. Antibiotic-eluting biodegradable polymeric coatings are a potential solution, but they require optimisation; an ideal drug release profile would consist of an initial 'burst release' followed by a prolonged release for several months, with the local drug concentration maintained above the minimum effective dose. This study aimed to characterise the *in vitro* release profiles and antimicrobial potential of rifampicin and poly(lactic-co-glycolic acid) (PLGA) coatings.

Methods

Three ratios of PLGA:rifampicin (50:50, 60:40, and 75:25) were dip-coated onto to stainless steel coupons and submerged in PBS at 37°C for 11 weeks. Elution was measured with UV-vis spectrophotometry (350nm light). Coatings of 100% rifampicin and 100% PLGA were included as controls.

Results

The percentages released from all coatings were significantly different for all timepoints up to 21 days ($p \leq 0.003$). Coatings with a lower ratio of rifampicin eluted more slowly: the 75:25 and 60:40 coatings continued to elute up to 11 weeks, while the 50:50 coating had released >99% of its total rifampicin by 7 days. At 24h, the 50:50 coating had released 79% (±14.0%), 60:40 had released 35% (±2.8%), and 75:25 had released 7% (±0.46%) of the total drug mass released. This demonstrated that coatings with a higher ratio of rifampicin yield a larger burst release. Preliminary work indicates that all coatings should provide sustained protection against bacterial colonisation.

Conclusions

Decreasing the ratio of rifampicin in PLGA coatings on stainless steel slows drug elution and minimises the burst release. This will inform further study to engineer an optimal rifampicin coating for prevention of infections in orthopaedic implants.

The role of Piezo1 in transducing matrix viscoelasticity

Mariana Azevedo Gonzalez Oliva, Oana Dobre, Massimo Vassalli, Matthew J. Dalby, Manuel Salmeron-Sanchez

Centre for the Cellular Microenvironment, University of Glasgow

Introduction

Local dynamic changes in tissue mechanics have been shown to exert a critical role in pathophysiological processes. These local mechanical cues are converted into biochemical responses via mechanotransduction. Piezo1 (P1) is a recently discovered mechanosensitive transmembrane ion channel found in various cell types, that relays mechanical stimuli exerted on cells to the nucleus. Although the channel is now associated with important physiological roles [1], the exact way by which it perceives the mechanical environment of cells and, in turn, mediates changes in cell and tissue behaviour is not fully understood. Therefore, the aim of this work is to understand the mechanism by which P1 transduces viscoelasticity intro transcriptional changes, as this has recently been shown to be key in relation to tissue function [2].

Experimental methods

Immortalised mechanosensitive mesenchymal stem cells (Y201 MSCs) [3] are used to investigate how P1 activity affects focal adhesion formation, cytoskeletal tension, YAP nuclear translocation and cell respiration on polyacrylamide (PAAm) substrates of controlled viscoelastic properties. P1 activity is regulated through the use of siRNA for P1 as well as GsMTx4 and Yoda1 molecules, which can block and activate P1 channels, respectively.

Results and discussion

P1 expression was knocked down in the Y201 cells through the use of siRNA; furthermore, channel activity was modulated in culture with the use of the P1 channel agonist Yoda1 and blocker, GsMTx4. Then, cell size, focal adhesions and YAP nuclear translocation were assessed in function of substrate dissipation (viscosity) (tan (Î´) of 0.175 to 0.4) of PAAm hydrogels as well as P1 channel activation. We observed that cell shape and size, focal adhesions, YAP nuclear translocation and cellular respiration were affected by the mechanical properties of the substrates [4]. However, when the P1 channel activity was blocked, this behaviour was inhibited, and vice-versa for when the channel was activated, alluding to the role of P1 as a key mechanosensor in cells for both matrix elasticity as well as viscoelasticity.

Conclusion

We have established a system in which to investigate the function of P1 in mediating cellular behaviour in response to physiologically emulating mechanical cues. As living tissues behave as viscoelastic solids, where both the elastic and viscous component is of relevance for cell proliferation and differentiation, this project will aim, in the future, to further investigate the role of P1 in transducing viscoelastic cues in cells.

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Engineering a bone marrow endosteal niche model for drug screening against Acute Myeloid Leukaemia

Lauren Hope [1,2], Alvaro Sanchez Rubio[2], Professor Helen Wheadon[1], Dr Catherine Berry[2], Professor Manuel Salmeron Sanchez[2], Professor Mhairi Copland[1]

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Acute myeloid leukaemia (AML) is the most common acute leukaemia in adults, accounting for 2% of all UK cancer mortalities (Cancer Research UK, 2021). AML is caused by the acquisition of mutations in haematopoeitic stem and progenitor cells, causing leukaemic stem cells (LSCs) to arise. Interactions between LSCs and bone marrow stroma within the endosteal niche induce LSC quiescence, protecting LSCs from chemotherapy. This leads to relapse in approximately 50% of patients. Circumventing this protective microenvironment is key to treating AML. Therefore, the aim of this project is to develop a 3D bioprinted co-culture bone marrow model to assess novel and current AML therapies. Alginate/gelatin hydrogels, of varied alginate composition, were selected due to their suitable printability and biocompatibility. Hydrogel structure was observed using Scanning Electron Microscopy (SEM), and stiffness was measured using rheology. Finally, cell morphology and viability of bone marrow fibroblast (HS5) spheroids and AML cell lines were assessed in hand-cast and 3D-bioprinted hydrogels. SEM revealed that the 1% alginate/8% gelatin gel had larger pores compared to the 2% alginate/8% gelatin, which corresponds to the reduced stiffness of 1% alginate/8% gelatin gels. Additionally, after two days in culture, there was no significant difference in stiffness between gels containing MOLM-13 AML cells and cell-free gels. Actin/DAPI staining indicated HS5 spheroids maintain their integrity within the gel, and that MOLM-13 AML cells were able to replicate within the gels. Additionally, calcein AM/Ethidium bromide viability staining showed that MOLM-13 cells remained highly viable within the gels for at least two days, further indicating that this gel is suitable for the model. These data indicate that 2% alginate/8% gelatin hydrogels would be most suitable for the model as spheroids maintained their compacted morphology, and did not migrate into the gel. Additionally, AML cells remained viable within the gels, further highlighting the suitability of this gel for use in developing an endosteal bone marrow model. When repeated in 3D-bioprinted alginate/gelatin hydrogels, there were no significant differences in viability and no disruption of spheroid morphology in the 3D printed gels, indicating that the printing process does not negatively impact the cells

POSTER ABSTRACTS

Cyclic hydrostatic pressure for osteogenic differentiation

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Bone is constantly exposed to a range of macro-scale loading, which generates a complex mechanical microenvironment for resident cells. One such a mechanical stress created is hydrostatic pressure (HP), which has an important role in cell function and fate determination. However, little is known about the effect of this external stimuli in a 3D microenvironment. Inspired by native bone mechanical microenvironment, this research investigated the effect of different ranges of cyclic HP on human adipose-derived mesenchymal stem cells (hASCs) encapsulated in a 3D liquefied microcapsule. Taking advantage of the liquefied core environment of microcapsules, hASCs were exposed to cyclic HP at 5 or 50 MPa magnitudes 3 times/week, at 37°C. Biological tests including fluorescence staining of F-actin filaments demonstrated a noticeable increase in cell-cell interactions and network formation of hASCs in the pressurized groups, compared to the non-pressurized group. Being this phenomenon more pronounced in OST condition, the observation confirmed by fluorescent staining of vinculin. More interestingly, a significantly higher alkaline phosphatase activity was detected in 50 MPa group. Furthermore, a greater staining of osteopontin, and hydroxyapatite markers was observed in 50 MPa/OST group. Overall, this study demonstrated that the proposed liquefied encapsulation system holds great potential as an effective platform for studying the impact of various magnitudes of HP for numerous differentiation purposes. Moreover, results revealed that the beneficial effect of HP for osteogenic differentiation is magnitude dependent. Finally, the highest differentiation effect was observed when both biochemical and mechanical cues were combined (50 MPa, OST).

Differences in male and female mice in a model of early osteoarthritis

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Osteoarthritis is a prevalent musculoskeletal disease characterised by joint degeneration and pain. Over the age of 50, women are twice as likely to develop osteoarthritis than men. Given the importance of biological sex in osteoarthritis, we compared early osteoarthritis in male and female mice.

We induced osteoarthritis via surgical destabilisation of the medial meniscus and cartilage scratch (DCS) on 10-week-old male and female mice (n=8). Two weeks after induction we characterised joint parameters via microCT and histology, and pain behaviours by dynamic weight bearing (DWB). We ovariectomised (OVX) 8-week-old females (n=10) and induced osteoarthritis at 11 weeks old. We assessed for pain behaviours via DWB and von Frey testing. Contralateral and ipsilateral dorsal root ganglion (DRG) of male, female sham OVX and female OVX were dissected and processed for gene expression RNAseq analysis.

Male mice showed 31.2 ± 4.5% increase in subchondral bone osteoclerosis while female mice showed a minimal 4.42 ± 6.23 % change in subchondral bone ($P < 0.001$). There were no significant differences in cartilage damage, synovitis or osteophytogenesis. Males loaded their osteoarthritic leg less than the contralateral (paired t-test $P < 0.01$), which indicates discomfort. None of the female mice or OVX female mice showed this change in loading. Female OVX mice showed a decrease in mechanical withdrawal threshold in the osteoarthritic leg (0.3 ± 0.12) when compared to the contralateral leg (0.5 ± 0.24, $P < 0.05$). There were no significant changes in DRG gene expression when comparing osteoarthritic to contralateral side in any of the groups. Female DRG show downregulation of genes involved neuron projection guidance and calcium ion transmembrane transport when compared to males. Female OVX mice showed an upregulation of lymphocyte development pathways when compared to female sham OVX DRG.

The main differences observed in early osteoarthritis between male and female mice resided in the bone and display of pain. Female mice in general have a slower bone modelling rate, which here translates into lower subchondral osteosclerosis. Interestingly, female mice did not display differential pain behaviours compared to male mice, which could be related to the gene expression changes observed in the DRG.

Optimising vibration parameters for osteogenic stimulation of MG63 cells

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The use of stem cells in bone therapies is quickly becoming realised as a desirable alternative to synthetic or xenogenic treatments. However in vitro, it is challenging to control and direct the differentiation of stem cells toward an osteogenic lineage without the use of chemical factors. The Universities of Strathclyde and Glasgow have developed devices capable of applying a continuous vertical nanovibrational stimulation at a 1 kHz frequency, 30 nm amplitude to cells in vitro, successfully increasing osteogenic response in mesenchymal stem cells (MSCs) without requiring chemical stimulation [1, 2]. However a more recent study has suggested that a higher amplitude of 90 nm may produce a higher osteogenic response from MSCs, suggesting that the optimal vibration conditions for inducing osteogenesis have yet to be found [3]. Other vibration studies have also found that horizontal vibration, in the plane of the cell monolayer, increases osteogenic response compared to vertical vibration [4]. Here we present data on the impact of vibration directionality and intermittent vibration on human osteosarcoma cells, MG63s, as an osteogenic cell line. A prototype device capable of applying nanovibrational stimulation at 1 kHz, 30 nm horizontally has been developed. Initial testing has included morphological (cell alignment, nuclear area and actin intensity) and gene expression analysis, comparing the effects of horizontal vibration to vertical vibration and the effect of intermittent (4 hours daily) vibration compared to continuous stimulation. Investigating vibration parameters further is hoped to allow engineering of targeted stem cell-based bone grafts as new advanced therapeutic products.

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Developing Magnetic Hydrogels for Bone Tissue Engineering

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Bone tissue engineering is used to generate lab-based replacements for bone tissue which has been damaged or needs replacement due to disease, following an accident or surgical excision. The strategy is to develop 3D structures which mimic the natural tissue in terms of the biological and mechanical properties, this then allows for cell growth, development, and differentiation into functional tissue. In this regard, hydrogels have an established track record as 3D models. Previous research shows magnetic stimulation can promote increased bone formation, allowing for a more rapid and better healing process.

Utilising a UV crosslinking gelatin hydrogel (GelMA), a magnetic hydrogel has been developed by incorporating superparamagnetic iron oxide nanoparticles into hydrogels. Properties of the model have been investigated including mesenchymal stem cell viability, hydrogel stiffness and contraction. Static magnetic fields (370mT) are being utilised to investigate the ability of magnetic actuation to accelerate cell proliferation, migration, and the differentiation of mesenchymal stem cells.

Utilising a GelMA concentration of 7.5%, investigations show increasing nanoparticle concentration reduces crosslinking ability of the hydrogel. Cell viability is maintained above 80% viability over 28 days. Ongoing investigations utilising a neodymium-iron-boron magnetic plate aim to optimise exposure time to magnetic actuation.

Development of a new natural polysaccharide-based hydrogel for bone tissue engineering

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The plant-derived polysaccharide has been reported that it possesses a high medical potential, immunomodulatory properties, and high potential for tissue engineering applications, such as wound healing and bone regeneration. As a dosage problem of growth factors delivery, the high dosage of GFs could lead a severe systemic side effect. To work on bone related application, we present a novel hydrogel which combined the benefits of acemannan and control release of growth factors. Fibronectin fragments with high affinity for GFs were added to the hydrogels to increase the sustained release of the GF. We believe that this novel hydrogel system will provide a controllable GFs release platform with the potential of boosting bone repair.

Controlling antibiotic release to counteract implant infection: a mathematical modelling approach

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Introduction

Implant infection is a serious clinical problem, with treatment usually involving systemic delivery of antibiotics. However, due to the ability of bacteria within biofilms to survive antibiotic dosages that would ordinarily kill free-swimming proliferative bacteria, biofilm infections are extremely difficult to eradicate. Antibiotic resistance and tolerance confound the problem, often associated with nutrient insufficiency, hypoxia in the deeper layers of biofilm and antibiotic concentration at levels above the Minimum Inhibitory Concentration (MIC) [1]. An alternative approach is to delivery antibiotics locally in a sustained manner. In this study, we present a mathematical model of biofilm growth subject to antibiotic delivery, with the aim of understanding how the biofilm growth and composition depends on the drug dose and release rate.

Methods

We have formulated a 1D biofilm growth model in which we introduce controlled antibiotic release directly from the implant. If the release is inadequate to prevent bacterial growth, then infection can take hold, however if drug release is excessive then this may impair the recovery of healthy tissue around the implant. This represents a delicate balance, amenable to exploration and optimization through mathematical modelling.

The approach of modelling biofilm growth while optimizing antibiotic dose and release rate simultaneously may result in a more efficient biofilm prevention strategy. The model consists of different bacterial phenotypes, self-produced extra cellular polymeric substance (EPS), nutrient concentration, water volume fraction in the biofilm pores, growth of the biofilm and a porous implant filled with antibiotic [2]. We have simulated how different model parameters, including nutrient concentration, influence the growth of different bacterial phenotypes. We also simulated how different antibiotic-release strategies from a nano-porous implant impact on the time-course of biofilm growth and its constitution [3]. In this model, antibiotic-induced death of active bacteria along with natural death are considered.

Results

As expected, the density of proliferative bacteria increases moving away from the implant, where antibiotic is being delivered from and decreases with increasing antibiotic dose. However, the persister bacteria, one of the main reasons for antibiotic resilience, increases with increasing antibiotic dose since the proliferative bacteria transforms into the persister phenotype in order to survive the antibiotic dosage.

Conclusions

Our model suggests that careful tailoring of antibiotic release could help prevent implant-associated infection as biofilm thickness and proliferative bacteria cells decrease with increasing antibiotic dosage. The model is able to capture experimentally observed resilience to antibiotic shown by persister cells. Our immediate next steps would be to find the optimal antibiotic delivery configuration such that the infection gets eradicated along with persister cells which will result in no further infections on the implant.

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Advanced Bio-active Coating for the Bio-Integration of Synthetic Vascular Grafts

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Introduction

Synthetic vascular graft failure is a persistent issue, that may be caused by anastomotic intimal hyperplasia, thrombogenesis and infection1. It has been widely suggested that in-vivo endothelialisation might result in high patency rates and reduced intimal hyperplasia2.

Here, we propose a bioactive coating - a material-based strategy for the local presentation of appropriate growth factors to promote healthy endothelialisation in vivo. Briefly, it comprises an elastomer coating functionalised sequentially with extracellular matrix protein, fibronectin, and selected stimuli, acting as the driving force for cell adhesion and migration.

Methods

The plasma polymerised PEA coating as well as the Fibronectin and Growth Factor(GF) layers (VEGF, FGF-2) were examined. A range of GF ratios (1VEGF:1FGF, 2:2, 2:1, 1:2) were investigated in terms of cell proliferation and cell-to-cell communication (CD31).

The in-house 3D-printed migration barrier inserts were validated before proceeding with cellular experiments.

Results

The efficiency of the coating procedures was shown through XPS for the PEA layer and ELISA's for the Fibronectin and GF Layer. Preliminary results show that cell proliferation was improved in certain conditions, which was also correlated with higher CD31 expression; specifically, when the system is loaded with 2:1, both cell proliferation and CD31 expression are improved. Finally, migration speed appears to be higher when stimulation included FGF-2.

Conclusion

The system appears to provide the conditions for increased cell proliferation, adhesion and migration. Further work is envisioned to showcase the ability of our system to promote the formation of a tight endothelial cell monolayer, moving from static to flow conditions, which will simulate physiological blood flow.

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Collagen-Acemannan hydrogels for wound healing applications

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Collagen is one of the most abundant proteins in the extracellular matrix of different tissues. It is highly present in skin and bones and has a crucial role in the regulation of the wound healing process. In addition, Aloe Vera has traditionally been used in skin care as well as to treat wounds. Acemannan, a bioactive polysaccharide derived from Aloe Vera, has shown to promote tissue repair. In the present work, hydrogels made of collagen and Acemannan are developed to explore their application in wound healing. Physicochemical characterisation of the hydrogels revealed suitable swelling, mechanical properties and Acemannan delivery to develop hydrogels for wound healing. Incorporation of fibroblasts into the hydrogels demonstrated their capacity to support cell viability and proved the ability of Acemannan to improve cell spreading and proliferation. Hence, these hydrogels could be a promising approach to develop novel biomaterials in the field of wound healing.

Fibroblast Culture in 3D Systems: A Comparative Study in Arthritis

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Inflammation is essential for responding to infections and subsequent tissue healing. However, chronic unresolved inflammation can become a serious health problem, as exemplified in the joints during Rheumatoid Arthritis (RA). Why does inflammation persist in RA? The answer could lie with Synovial Fibroblasts (SFs), non-haemopoietic cells that can adopt a pathogenic phenotype that fuels disease progression for years. Critically, targeting SFs could stop joint inflammation without suppressing systemic immunity. Nevertheless, such basic research findings have not been translated to new drugs, perhaps because non-physiological may have been generated in 2D cultures.

We employed RNAseq, imaging and functional assays to investigate how various culture systems modify synovial fibroblast biology ex vivo. We used freshly isolated SFs from murine synovium, both from healthy and animals undergoing experimental arthritis, and we compared their activated phenotype with cells expanded in conventional 2D cultures and in two unrelated 3D systems: i) fibronectin-(FN)-coated polystyrene scaffolds and ii) fibronectin-(FN)-based 3D hydrogels. Scaffolds were commercially available, and hydrogels were produced in-house, functionalizing the FN protein with PEG-maleimide (PEGylation).

Our results revealed that on 3D scaffolds, perhaps mimicking the joint/bone environment, the cells partially recovered their pathogenic phenotype indicating that this may provide a more suitable platform for candidate drug screening. Intriguingly, we found their culture in biogels to induce a 'remission-like' phenotype that may offer therapeutic potential. These novel findings and their clinical implications will be of interest not only to rheumatologists, but also scientists researching stromal biology and other chronic inflammatory diseases where fibroblasts play a key role in disease progression, such as colitis, asthma or cancer.

Harnessing Stem Cell Response to Viscoelasticity

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The cellular microenvironment is a mechanically complex, dynamic niche that exhibits time-dependent responses to deformation. Despite this viscoelastic behaviour, materials utilised in studies of cellular mechanotransduction are generally purely elastic and don't recapitulate the true mechanical environment experienced by cells in vivo. Recent research has investigated the role of viscoelasticity on cellular behaviour; however, conflicting results have been reported, highlighting that our knowledge in the field is still limited. Here, we aim to address these shortfalls in knowledge in mechanobiology by developing robust material platforms with fully controllable viscoelastic properties, and by investigating the mechanotransductive mechanisms of cell response. We have developed and characterised a family of isoelastic polyacrylamide hydrogels with finely tuned viscous properties (same Young's modulus, different loss modulus) within a physiologically relevant range. We have functionalised these hydrogels with the protein-derived adhesive ligands RGD (derived from fibronectin) and IKVAV (derived from laminin) in order to determine the integrin-dependent response to material viscoelasticity. We have utilised a range of biophysical techniques (e.g. single cell force spectroscopy, traction force microscopy, atomic force microscopy) and biomolecular techniques (e.g. quantitative PCR, immunofluorescence microscopy) to derive how stem cells respond to a viscoelastic environment. We found that hMSC mechanoresponse to viscoelastic interactions depended on the hydrogel stiffness. Specifically, increasing the viscosity of soft hydrogels (<1kPa) enhanced cellular adhesion, traction and spreading, while the opposite trend was observed on stiffer hydrogels (>3kPa), independently of the ligand. Furthermore, we observed mechanoactivation of Yes-associated protein (YAP) in spread cells on rigid elastic hydrogels, particularly in the presence of RGD, but not in cells spread on soft viscoelastic hydrogels. This indicates a mechanical disconnect between cellular traction and nuclear mechanotransduction on soft viscoelastic hydrogels. Ultimately, the differential activation of mechanosensitive pathways altered lineage commitment. Our results demonstrate the dependence of hMSC mechanoresponse to viscoelasticity on stiffness and ligand type. Overall, we establish a paradigm of stem cell response to viscoelasticity, presenting an avenue for the design of next generation viscoelastic biomaterials for mechanobiology and regenerative medicine.

Developing an off the shelf stem cell 'niche'

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In the niche, stem cells are regulated to maintain a naive phenotype. In this project, we aim to bioengineer 3D printed 'off-the-shelf' niches using hydrogels that support cell viability in 3D coupled with use of bioactive metabolites. We are studying marker expression and niche-relevant cytokine secretion to assess mesenchymal stem cell (MSC) phenotype on extracellular matrix (ECM) proteins; laminin (LM) and fibronectin (FN), absorbed onto poly(ethyl acrylate) (PEA) coated tissue culture plates (TCP). PEA directs opened conformations of ECM proteins, exposing integrin and growth factor binding sites.

Methods

LM and FN were absorbed onto PEA coated 24 well plates and 10cm tissue culture dishes, and In-cell western and Western blotting utilised to measure MSC protein expression for markers of primitive phenotype; Nestin, CD166, Tom 20, TPO, SDF and SCF. Cells were treated with Brefeldin A, a protein transport inhibitor which prevents protein secretion, to increase protein concentration, signal intensity and aid subsequent image analysis.

Results and discussion

Both ECM proteins resulted in increased cell spreading and proliferation compared to non-treated TCP. ECM proteins also enhanced protein expression compared to TCP, indicating a more favourable environment for the cells. Next steps will be the addition of niche-like growth factors to determine if MSC phenotype can be increasingly retained in culture before introducing collagen hydrogels to further improve the niche-like properties of the model. Subsequently, metabolomics will allow us to observe activity metabolites depleted as MSCs maintain their phenotype, and then utilise these in 3D printed models using Atelerix bead ready alginate hydrogels.

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Bioengineering of a model of the bone marrow niche to investigate the mechanical changes in the microenvironment

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The bone marrow niche contains a multitude of cell types and a microenvironment supportive of haematopoiesis. Mesenchymal stem cells (MSCs) secrete various growth factors which support the maintenance of haematopoietic stem cells (HSCs) in a quiescent state. Similarly, leukemic stem cells rely on these interactions in the bone marrow niche to support their survival and resistance to treatment. Improvements in the culturing of HSCs and MSCs in vitro have involved (1) the development of 3D culture models; (2) the co-culture of the two cell types; and (3) the addition of mechanical stimulation.

Here, we have designed and optimised a system to recapitulate the 3D microenvironment of MSCs and HSCs by using synthetic fibronectin-based hydrogels in which the stiffness and degradability can be tuned. Mechanical cues have an underappreciated role in fostering the correct functioning and survival of cells, therefore, the effect of applying mechanical stimulation to the model niche was explored by applying vibrations of nanoscale amplitude and measuring the bulk and local mechanical properties of the hydrogels.

We show that MSCs, HSCs and LSCs are viable when cultured in our 3D model of the bone marrow niche. Further, we demonstrate that HSCs and leukemic stem cells respond differently to mechanical stimulation. In conclusion, we have developed a method for the 3D culture of the bone marrow niche incorporating both HSCs and MSCs which can be utilized to investigate the mechanical changes in cell and ECM stiffness in response to malignant cells.

Modelling the Bone Marrow Niche using PEG-based Viscoelastic Hydrogels

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The bone marrow (BM) niche is a specialised environment that provides appropriate cues for Hematopoietic stem cell self-renewal and differentiation(1). Its extracellular matrix (ECM) is not purely elastic but exhibits complex mechanical behaviour in response to mechanical loading, such as stress relaxation and creep(2). However, efforts to understand the role of ECM viscoelasticity in regulating cell behavior have only recently been made using 3D tissue-model hydrogels(3). Here, we propose a novel approach to develop 3D stiffness-dependent families of ECM-resembling hydrogels, each of which consist of hydrogels with similar stiffness but varying stress relaxation rates.

Polyethylene glycol-Maleimide (PEG-MAL) hydrogels were prepared using Michael-type addition reaction and functionalized using full-length Fibronectin, a protein abundantly found in the BM ECM. Viscoelasticity was accomplished by altering the branching and molecular weight (MW) of the macromers and crosslinkers. The bulk mechanical properties including the storage modulus (G) and stress relaxation were measured using a stress-controlled rotational rheometer.

8-arm and 4-arm PEG-MAL of different MWs were combined with crosslinkers of different MWs to create synthetic viscoelastic hydrogels. We observed that through different macromer and crosslinker combinations we could create stiffness-dependent families of hydrogels (Family 1 = soft gels, with G of ~250 Pa & Family 2 = stiff gels, with G of 2 kPa) consisting of hydrogels with different viscosities and stress relaxation rates. Furthermore, we observed that more than 80% of human BM-Mesenchymal stem cells were viable after 7 days in all hydrogels.

We have developed a system through which we can modulate the viscosity and stress relaxation of PEG-based hydrogels independently of the elastic modulus. These can be functionalized with ECM proteins to resemble the native BM ECM. The development of such biomaterials may advance our understanding of the healthy/ diseased BM niche.

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Management of de Quervain's tenosynovitis: a living systematic review and network meta-analysis of randomised studies

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Background

The aim of our study was to present the highest quality evidence on the management of de Quervain's tenosynovitis (dQt) and perform a comparison of interventions to guide clinical practice and future guidelines.

Methods

Literature searches were conducted in August 2022 in multiple databases aiming to identify all randomised controlled trials (RCTs) assessing the effectiveness of any intervention for the management of dQt. Pairwise and network meta-analyses were performed for pain (visual analogue scale, VAS) and function (quick disabilities of the arm, shoulder and hand, q-DASH scale) for short-term (0-12 weeks) and mid-term (13 weeks-12 months) follow up. Mean difference (MD) and odds ratios (OR) with their 95% confidence intervals (CI) were calculated for the pairwise meta-analyses. The Cochrane Risk of Bias (RoB 2) tool and the GRADE tool were used for risk of bias and certainty of evidence assessment for each outcome.

Results

A total of 29 studies (1611 patients) were included in our systematic review, of which 19 participated in quantitative analyses. From the pairwise meta-analyses, based on evidence of moderate certainty, adding thumb spica immobilisation for 3-4 weeks to a corticosteroid injection (CSI) is associated with statistically but not clinically significant short- and mid-term functional benefits [MD q-DASH 10.5 points CI (6.8, 14.1) and 9.4 points CI (7.0, 11.9)]. This was also associated with statistically but not clinically significant short- and mid-term pain relief benefits [MD VAS 1.3 points CI (0.4, 2.1) and 1.2 points CI (0.3, 2.2) respectively], and, additionally, ultrasound-guided CSI was superior to conventional CSI for short-term pain at clinical but not statistical significance [MD VAS 2.1 points CI (-0.5, 4.6)], however these results were based on very low certainty of evidence. For open surgical release, transverse skin incision was associated with a greater incidence of total complications and nerve injury compared to longitudinal incision [OR 6.8 CI (0.9, 48.1) and OR 7.7 CI (0.9, 64.0)] but these did not reach statistical significance. In the network meta-analysis, interventions that included ultrasound-guided CSI ranked at the top for pain. CSI with thumb spica immobilisation had the highest probability to be the most effective intervention for function. Placebo injection (normal saline) and thumb spica immobilisation alone (splint or cast) were shown to be the least effective interventions.

Conclusions

Until high-quality evidence suggests otherwise, we recommend the use of CSI with thumb spica immobilisation as first-line treatment for dQt patients. We also present our recommended treatment pathway.

Engineering obesity microenvironments to investigate breast cancer progression, invasiveness and recurrence

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According to epidemiological studies, obesity is considered as a high-risk factor for breast cancer, and it is linked with higher mortality rates and increased levels of recurrence compared to lean individuals. Recent studies have also shown that obesity promotes tumour initiation and progression by providing not only a suitable microenvironment with specific mechanical and architectural cues but also an altered biochemical signalling, which together further support tumour growth. Nevertheless, the precise effect of obese tissue microenvironment and cell content on invasiveness and recurrence of breast cancer has not been evaluated yet. The extracellular matrix (ECM) is a key fibrillar/scaffolding component of tissues, and it was reported that ECM composition, microarchitecture and mechanics all play essential roles in the cross-communication and spatial interaction between obese and cancer tissues. In this study, we have used temperature casting to fabricate obese-mimicking and control/lean collagen scaffolds with tuneable/controlled microarchitecture and mechanics. The interaction between those obese/lean microenvironments and cancer cells has then been investigated by monitoring both the growth (and invasion) of embedded tumour spheroids and the associated mechanical and structural alterations of the surrounding collagen/ECM scaffolds. Our preliminary data clearly show that cancer cells invasion primarily depends on matrix architecture (mesh size and fibre thickness). These findings could improve our understanding of the link between obesity and breast cancer metastasis, recurrence, and mortality rates

Bioengineering leukemic niches to develop stem cell therapies

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Despite advances in therapeutics and early detection screening methods, acute myeloid leukaemia (AML) is characterized by high mortality rates. Patients with AML that are relapsed or refractory for standard chemotherapy can benefit only from allogenic haematopoietic stem cell transplantation (HSCT). However, 50% of HSCT is unsuccessful due to graft rejection by the recipient while bad responders experience very poor survival outcomes. Importantly, there is no available mouse model that can mimic the human leukemic microenvironment comprehensively. Hence, it can be suggested that different models are required to study the progression of the disease and efficiency of novel therapies. Tissue bioengineering is an emerging field that has conferred alternative approaches for mimicking disease development and progression. The development of humanized models has the potential to resemble the tissue microenvironment more reliably while also to incorporate primary patient cells. Noteworthy, MSCs can be of great use in 3D model bioengineering due to their regenerative properties. In this project, we aim to use MSCs to establish a BM leukemic niche as well as exploit their regenerative, anticancer, and immunosuppressive properties which will result in a combined cellular therapy to enhance HSC maintenance.

Towards the development of a hydrogel-based cartilage 3D model for osteoarthritis disease

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Introduction

Osteoarthritis (OA) is a degenerative joint disease characterised by pain and progressive deterioration of articular cartilage and bone. Prior preclinical testing resulted to be a poor predictor of drug efficacy in clinical trials, but 3D tissue-mimic models are a promising approach to study disease progression and improve drug screening. To facilitate OA studies, our work focused on the development of a hydrogel-based 3D model mimicking cartilage tissue, using alginate as a biomaterial scaffold, and tested its ability to support chondrogenic differentiation of human mesenchymal stem cells (hMSCs) and chondroprogenitors derived from induced pluripotent stem cells (iCPs).

Materials and methods

Sodium alginate hydrogels properties were analysed with an Anton Paar MCR302 rheometer. Cell viability inside gels was determined with Live/Dead assay (Invitrogen). Chondrogenic differentiation of stem cells and mesodermal differentiation of human iPSCs were performed as previously reported. Gene expression was evaluated with qPCR, and alcian blue staining on paraffin sections was used to verify proteoglycan deposition.

Results

1-3% (w/v) sodium alginate solutions were used to create hydrogel discs with an initial storage modulus (G') ranging from 3 to 20 kPa. Gels placed in cell culture media showed decreasing G' and tanδ. Gels relax almost completely during stress relaxation tests. Stem cells inside gels show good viability. Preliminary studies suggested that MSCs in softer gels show enhanced expression of chondrogenic markers (SOX9, COL2, AGG, COL1, COL10). To confirm these findings, we performed chondrogenesis of human MSCs and iCPs in 1-3% (w/v) alginate gels and found that hMSCs in 2% (w/v) gels show higher chondrogenic gene expression and proteoglycan deposition. iCPs do not show efficient proteoglycan deposition in either concentration.

Conclusion

Alginate hydrogels are biocompatible, soften with time and can be plastically remodelled. Preliminary results with mMSCs corroborate previous findings suggesting that softer alginate gels better support chondrogenic differentiation of stem cells. Chondrogenesis of hMSCs in soft gels suggests the 2% gel percentage as more suitable for differentiation, while iCPs may need additional cues to effectively differentiate. Future studies will include alginate gels modification to develop a more tissue mimicking biomaterial.

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Upscaling the nanovibrational cell production process with microcarrier suspension culture

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Nanoscale amplitude sinusoidal vibration (1kHz, 30nm) delivered continuously in a pistonic manner by a nanovibrational bioreactor (1), drives the differentiation of mesenchymal stem cells (MSCs) towards an osteoblast lineage, further, osteogenesis has been confirmed by the presence of mineralisation (2). Bone is the second most transplanted tissue, as such, there is a great demand for high-quality bone tissue and osteoregenerative interventions. Original versions of the nanovibrational bioreactor - built for proof-of-concept experiments - were designed to nanovibrate individual Petri dishes. Upgrades enabled the bioreactor to deliver consistent vibration to standard flasks up to T150 in size. MSCs are adherent, therefore, the surface area is the key parameter when considering upscaling the nanovibrational cell production process towards production of cellular therapies for orthopaedic use. Large surface area increases - with almost unbound potential - come with microcarrier suspension culture systems. In this study, we are performing proof-of-concept experiments to determine the efficacy of nanovibrating microcarrier spinner flasks to future-proof the process for supply of osteogenic cells to large patient groups.

An experimental setup designed to deliver nanoscale vibration to MSCs - adhered to microcarriers (polystyrene beads with diameters 125-121 µm) suspended in a 250 mL spinner flask by a rotating impeller - was developed, optimised, characterised, and tested. In initial proof-of-concept experiments MG63 cells were cultured in the vessel for up to 2-weeks. The nanovibrational cell production process will be assessed using different techniques, including, RT-qPCR, immunofluorescence, alizarin red, and micro-computed tomography. Soon, cell culture with MSCs will begin.

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ABSTRACTS ONLY

The interplay between matrix viscoelasticity and cell shape in regulating cell behaviour

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Evidence is growing that extracellular matrix (ECM) viscoelastic properties, in addition to elasticity, are a key determinant of the pathophysiological response of cells. Seminal studies showed that substrate stress relaxation mediates important processes such as cell spreading and differentiation, but our understanding of how ECM viscoelasticity mediates mechanobiological processes is still elusive. Cells in the ECM are not only influenced by the surrounding mechanics, but neighbouring cells and distribution of ligands impose spatial constraints that shape cell geometry, also regulating fundamental processes such as cell proliferation, apoptosis and differentiation.

Because of this, to recapitulate the complexity of the cellular microenvironment, viscoelastic cues should be combined with geometrical stimuli.

In this work, viscoelastic polyacrylamide hydrogels were micropatterned with fibronectin using a maskless photolithography approach (light induced molecular absorption) to study cytoskeletal and nuclear organisation, adhesion formation, YAP translocation, actin retrograde flow and cell mechanics in biomimetic microenvironments recapitulating the complexity of the ECM. This work will elucidate the combined role of matrix viscoelasticity and cell shape in regulating mesenchymal stem cell behaviour.

Early versus delayed mobilisation for non-surgically treated proximal humerus fractures: a systematic review and meta-analysis of randomised trials

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Background

Proximal humerus fractures (PHFs) are among the commonest bony injuries and the majority of them can be managed non-surgically. The aim of our systematic review and meta-analysis was to compare the effectiveness and safety of early versus delayed mobilisation in conservatively treated PHFs.

Methods

A literature search was performed in multiple databases in February 2023 aiming to identify all randomised controlled trials (RCTs) comparing early versus delayed (conventional) mobilisation as part of the non-surgical management of PHFs. Primary outcomes were patient-reported function and pain at short-term (3 months), mid-term (6 months) and long-term (12 months) follow up, and secondary outcomes included secondary fracture displacement and total complications. Meta-analyses produced mean differences (MDs) or standardised MDs (SMDs) for continuous outcomes and odds ratios (ORs) for binary outcomes, with 95% confidence intervals (CI). Certainty of evidence was assessed using the GRADE tool. Recommendations for clinical practice were given only based on results of high or moderate certainty of evidence.

Results

Six (6) RCTs were included that compared early mobilisation (EM; within one week from injury) to delayed mobilisation (DM; after 3 or 4 weeks of immobilisation). There were no differences in patient-reported function (combined or Constant score) or pain between the EM and DM groups at any follow up time points except for a significant difference in combined function scores favouring EM [SMD 0.4 CI (0.1,0.7), P=0.006]. There were no significant differences in the incidence of secondary fracture displacement and total complications in the two groups [OR 3.5 CI (0.7,18.2), P>0.05, and OR 1.2 CI (0.5,2.9), P>0.05, respectively]. All results were based on moderate or high strength of evidence.

Conclusions

We recommend commencement of early rehabilitation for non-surgically managed PHFs after a short period of immobilisation for no more than one week, which should be prescribed purely for comfort.